DEVELOPING AN UNDERSTANDING AND IMPROVED SENSORY QUALITY OF LOW ALCOHOL BEER

By Imogen Ramsey

Thesis submitted to the University of Nottingham for the degree of Doctor in Philosophy, September 2020

Acknowledgements

Firstly, I would like to thank my sensory supervisors Rebecca Ford and Qian Yang, who have both been incredible sources of knowledge and support throughout the process. I have been very lucky to have you both as my supervisors, and can always count on you for not only constructive feedback, but also a friendly chat. Thank you for everything you have done for me over the years, whether it be checking through conference presentations, or supporting me during the tougher times of the PhD. I would also like to thank Ian Fisk for his guidance throughout the membrane filtration work and flavour analysis, whilst also welcoming me into the Food Flavour group.

In addition, I would like to express my gratitude to Joanne Hort for taking a chance on a girl who was being interviewed in a hostel room in the middle of South America and who, when asked what her favourite beer was, replied ‘whatever is cheapest’. Thankfully, my tastes have become a lot more refined during the 4 years! The guidance you gave me during the first year of the PhD ensured I continue to question science every step of the way. In addition to this, I am also very grateful for the help and guidance from Carolyn Ross during my first year, guiding me on planning my first experiment and writing my first paper. The combination of both of your extensive knowledge, and passion for sensory science, inspired me to learn and put me in good stead for the following years.

Finally, I would like to thank the Technical staff and both the Sensory Science and Food Flavour groups within the university, as well as my funding bodies: BBSRC and Campden BRI for their support during the 4 years.

To my parents, brother and extended family (Granny, Grandpa, Rob, Sarah, Matt and Ellie), I would like to thank you for your support and interest in the project - from Christmas sensory tastings of some pretty disgusting beers, to being dragged around Mindful Drinking festivals in London, I am forever grateful.

To everyone past and present within both BABs and Food Science who have made this journey a little bit easier by listening, laughing and going for pints. Especially I would like to thank Marit, James, Eoin, Marcus, Celina, Neil and Saskia. You have kept me (in)sane during the years with hilarious lunchtime discussions, amazing holidays and enjoyable conferences. Finally, a special thanks goes to Chris for encouragement and support throughout the process, and always making me laugh through the highs and the lows. Behind every great woman, is an even better man making incredible sandwiches for lunch!

Table of Contents

Abstract

The international non-alcoholic beer (NAB) market is predicted to be worth over $25bil by 2024. Consumers across the globe are limiting their alcohol consumption due to changes to healthier lifestyles and increased knowledge of long-term effects of alcohol. Research has shown however, that consumers find NABs ‘bland’, ‘disappointing’ and ‘less tasty’ than their higher alcohol counterparts. Consequently, the development of a NAB that displays similar sensory pleasure to its higher alcohol counterpart is an attractive proposition to manufacturers and consumers alike. This research therefore aimed to understand both the sensory and physicochemical role of ethanol in beers with different ethanol concentrations and within a range of commercially produced NABs, whilst also identifying the overall effect on consumer liking. Furthermore, investigations into the effect of physical dealcoholisation techniques (namely reverse osmosis) on the sensory and physicochemical properties of two different beer styles were assessed. To achieve these aims, four studies were employed.

Consumers indicated their liking and changes in temporal sensory properties for flavour, taste and mouthfeel in beers with different ethanol concentrations. No significant differences amongst samples were discovered for overall liking; however cluster analysis revealed three groups of consumers with different liking patterns. Drivers of liking/disliking were discovered for each cluster, highlighting that in relation to ethanol concentration, different negative and positive sensory drivers of preference exist for different segments of consumers. Overall, ethanol was shown to be linked to the perception of

sweetness, mouthfeel/body and alcohol warming sensations by consumers. Furthermore, differences in retronasal flavour by consumers, such as increased maltiness in the 0% beer, led to investigations exploring saliva*ethanol interactions as the mechanisms, with results showing that ethanol had a subtle inhibitory effect on binding of hydrophobic compounds to α-amylase. This thereby increased their headspace concentration in higher ethanol concentrations. This research provided a basis for further investigations in the reformulation of NAB.

To further explore the effect of reduction of ethanol, physicochemical and sensory differences amongst commercial NABs were reported. Different clusters of samples were found, yet specific production methods were not able to fully explain these clusters; instead other important pre and post processing methods were proposed to be the reason for this. On a broader level, grouping production methods into physical or biological techniques suggested that physical production methods produced beers with undesirable sensory characteristics, such as bitterness and astringency which were least liked by consumers. However, physical methods, in particular membrane filtration techniques, have been reported by the literature as being the most promising for producing NABs with least volatile reduction, yet few studies looking at the effect this process has on sensory properties have been conducted. Therefore, improved understanding of these techniques was gained through sensorial and physicochemical analysis by the dealcoholisation of both a lager and stout style beer using a selected membrane - reverse osmosis. This technique significantly impacted the overall quality of the beer, due to extreme losses of volatile flavour compounds which affected sensorial characteristics, identified by a

trained panel. Volatile losses were proposed to be due to volatile structure, as opposed to size as proposed by the literature. Furthermore, replicate trials found decreased efficiency in running times, proposed to be due to membrane clogging, and a presence of a contamination residue within the dealcoholised beer, suggested to be the result of membrane fouling.

This research delivered valuable insights on the sensorial and analytical influence of ethanol concentration, advancing the little published data available on the impact within a beer matrix. For the first time, the in-depth assessment of commercially produced NABs revealed that advancements in technologies meant that sensory profiles can be altered by pre and post processing methods. Reverse osmosis severely impacted the sensory quality of different beer styles, showing more research is needed to improve understanding of products produced by this method. This thesis furthers understanding of both sensorial and physicochemical characteristics of NABs, providing insights for the successful development and reformulation of NABs with desirable sensory characteristics.

1 General Introduction

General Introduction

The research presented in this thesis focuses on gaining an understanding and improved sensory quality of non-alcoholic beer (NAB). Before elaborating on the experiments conducted, a brief overview of the beer market, its components and the brewing process is presented as a basis for further sections reviewing the NAB market and associated production methods. Furthermore, a review of the physicochemical and sensorial effects of ethanol highlights its complexity. The role of sensory in assessing products provides a basis for the methodology presented in the research chapters.

1.1 Beer and Components

The global alcoholic drinks sector produces 257 billion litres per year, with beer contributing to the majority of this at 201 billion litres (Euromonitor, 2020). Alcoholic drinks in the UK reached a total market value of £48 billion in 2018, with wine and lager found to be the leading products in this sector and therefore the most popular types of alcoholic beverages (Mintel, 2019a). Focusing on the beer market, expected growth is predicted to be around 16.4% over the next five years to 2024, reaching a market value of £22 billion. Lager dominates the beer market, accounting for three quarters of the value and volume sales (Mintel, 2019c).

The four main ingredients in beer include water, malted barley, hops and yeast. These ingredients are fixed in Germany by the Purity Law, which is legislation governing commercial brewing; however other countries may use a mix of different grains known as adjuncts, which can include wheat, rice, rye

and spelt. The brewing process includes wort preparation (mashing), wort boiling, fermentation, maturation and filtration or stabilisation (Briggs et al., 2004). Mashing is a complex physical, chemical and biochemical process and is one of the main steps in beer production. During the process, starch is degraded by the help of enzymes; α-amylase degrades fermentable sugars and dextrin, whilst β-amylase further degrades dextrin into maltose. Changes in the mashing process therefore can determine the amount and type of sugars present, fermentability of the wort and thus alter the final ethanol concentration of the product (Branyik et al., 2012, Güzel et al., 2020). Wort boiling then occurs, which removes the possibility for contamination within the beer, but also allows for the addition of hops, which contribute to aroma, flavour and bitterness (Briggs et al., 2004). Yeast is then added, which converts these sugars into beer by producing ethanol, volatile flavour compounds and carbon dioxide during fermentation (Briggs et al., 2004). It is well known that beer is a complex product made up of over 450 volatile and non-volatile components which are responsible for giving beer its characteristic profile, with the majority of these components developed during fermentation (Briggs et al., 2004). The volatiles such as higher alcohols, esters, aldehydes and organic acids are responsible for the aroma and flavour of the beer, and the non-volatile components, including: inorganic salts, sugars, amino acids, nucleotides, polyphenols, hop resins, polysaccharides, proteins and nucleic acids, impact taste and mouthfeel (Briggs et al., 2004). The beer is then held for maturation, or secondary fermentation, to refine the flavour.

Brewing is one of the oldest known biotechnological processes, which has been finely-tuned over thousands of years. Therefore a small change in the

process, such as limiting ethanol production to produce NAB, can significantly alter the sensory properties of the finished product.

1.2 Non-Alcoholic Beer and Low Alcohol Beer (NABLABs)

NABLABs have been experiencing increased interest and innovation in the past few years due to many factors, which will be discussed in further sections. However, many countries have different definitions for beers with lower alcohol contents, making terminology confusing.

1.2.1 Definitions

There is no uniform declaration of conformity across the globe in terms of low alcohol descriptors, with common confusion amongst both industry experts and consumers. Even within Europe there are discrepancies, with the EU regulation no. 1169/2011 stating only that the alcohol content must be declared for drinks with an alcohol content above 1.2% vol (European Parliament and Council, 2011). Table 1.1 shows a comparison of a range of selected countries and their labelling requirements for beers with varying ethanol concentrations.

In 2018, the labelling regulations in the UK were to expire, which led to lobbying by craft industry members to change the laws surrounding these descriptors (Department of Health & Social Care, 2018b). Confusion was shown with the fact that products in the UK could only be labelled ‘alcohol free’ if they were ⩽0.05% ABV, yet EU manufactured products could be sold to the UK market below 0.5% ABV with an ‘alcohol free’ label (Department of Health & Social Care, 2018b). The term ‘dealcoholised’ was also called to be removed, as they believed not only was this term confusing for consumers, but

also meant that many of the products made in this category did not fit the definition. This is because many of the newly launched products do not undergo an alcohol removal technique, and instead are made using altered brewing methods (Department of Health & Social Care, 2018b). However this change was not passed, with the Department of Health saying there was 'no compelling new evidence' to suggest a change was needed to current low-alcohol labelling (Department of Health & Social Care, 2018b).

Therefore, for the purpose of this thesis the term 'non-alcoholic beer' (NAB) will be used, as 'alcohol-free' shows discrepancies amongst countries. Thus the definition of NAB used will be the same as in the USA, with alcohol levels ⩽0.5% ABV.

Table 1.1: *Descriptors and maximum ABV values for beers with varying ethanol concentrations in different countries (edited from Montanari et al. (2009))*

Definition	UK	Spain	Germany	Belgium	USA
Low Alcohol	⩽1.2% ABV	1-3% ABV	>0.5 and ⩽1.2% ABV	>0.5 and ⩽1.2% ABV, gravity ≥22°P	<2.5% ABV
Dealcoholised	Alcohol extracted, <0.5% ABV	-	-	-	-
Non-Alcoholic	Sacramental wine only	-	-	-	⩽0.5% ABV
Alcohol Free	⩽0.05% ABV	⩽1% ABV	⩽0.5% ABV	⩽0.5% ABV, gravity ≥22°P	0.0% ABV

1.2.2 Market Value, Consumer Perceptions and Reasons for Limiting Consumption

Although the global beer market has steadily been declining over the years, the global NAB market has been increasing and was valued to be worth $13.5 billion in 2016. This is expected to grow annually and be worth $25 billion in 2024 (Ahuja and Rawat, 2019). Similar to regular beer, lager is the leader in the low/no alcoholic segment with a reported value of £50 million in 2018/2019 (Mintel, 2019b). With 52% combined global volume, the category was led by markets in Iran, Germany, Spain and Nigeria in 2016 (Euromonitor, 2017c). The Middle East markets are the largest as alcohol consumption is strictly prohibited due to religion, with NAB the only beer permitted in this market (Euromonitor, 2017c). Nigeria, on the other hand, holds the fourth largest market for NAB, due to high consumer demand for malt beverages (Euromonitor, 2017c). In the EU, 60% of Spanish beer buyers purchased NAB, whilst in Germany one in five beer launches were non-alcoholic in 2013 (Mintel, 2014). Interestingly, the USA market is the only market that has not experienced growth in this sector over the past five years (Liguori et al., 2018b). In the UK, NAB sales recorded the strongest total volume growth within the beer sector, with a 29% increase between 2013 and 2018, and sales of 101 million litres (Euromonitor, 2019a). This is expected to grow another 20% between 2018 and 2023 (Mintel, 2019b).

Consumer perception of NABLABs have shown concerning results for the industry, with both consumer studies and market research reports stating that consumers find lower alcohol alternatives to be ‘bland’, ‘disappointing’ and ‘less tasty’ (Chrysochou, 2014, Mintel, 2015, Porretta and Donadini, 2008,

Silva et al., 2016). ‘Lacking in choice’, ‘artificial’ and ‘poor value for money’ were also cited (Mintel, 2015). In addition, consumers reported that the factors which would encourage them to choose to drink low or no alcohol alternatives over their standard-strength equivalents included: ‘similar taste’, ‘cheaper’, ‘fewer calories’ and ‘wider availability in pubs/restaurants’(Popper et al., 2004). In consumer studies, two groups of consumers were found with different drivers of motivation for consumption of NAB. One group cited ‘flavour’ as the most important attribute, whereas another group did not choose NAB for its flavour and instead due to wanting to ‘avoid alcohol’ (Silva et al., 2016). Consumers under 35 were found to be the most likely to link non-alcoholic beverages with positive attributes, as they associated them with ‘high quality’ and ‘interesting flavours’. Drinkers over the age of 55 however, viewed these drinks as ‘artificial’ and stated there is a ‘narrow range of choice’ (Mintel, 2015).

Reasons for the growth in the NABLAB sector can be attributed firstly to the increased concern on health and wellbeing, with a growing number of health conscious consumers limiting their alcohol consumption (Euromonitor, 2019a). According to research conducted by Mintel (2019b), one fifth of adults do not drink alcohol, with a third limiting their alcohol consumption. 47% consumers cited ‘health improvements’ as a reason they had limited/reduced alcohol intake, with 38% also wanting to ‘manage their weight’ (Earthy et al., 1997). 34% consumers also cited that they had limited/reduced alcohol consumption to ‘save money’. Other reasons included ‘avoiding a hangover’, ‘improving appearance’, ‘giving up alcohol on a particular month (e.g Dry January, Go Sober for October)’ and ‘staying within NHS/government

guidelines' (Mintel, 2019b). In consumer studies it was concluded that light beer and low-alcohol beverages are considered more as a healthy alternative, and less as a substitute for regular alcoholic beverages (Chrysochou, 2014).

Another reason for increased growth in this sector, is due to industry leaders committing to responsible drinking targets, guaranteeing over the next few years to tackle the rise in alcohol-attributed issues by releasing new products to market. AB InBev has promised that by 2025, 20% of their global beer volume will consist of no or lower-alcohol alternatives, whilst also pledging to spend $1 billion on marketing campaigns to encourage smarter drinking behaviour (ABInBev, 2018). Heineken highlighted innovation in low and no alcoholic drinks amongst its sustainability commitments (Heineken, 2019). Carlsberg has committed to 'zero irresponsible drinking' by 2030 (Carlsberg, 2019), and Molson Coors aims to offer more choice in the low/no alcohol sector by 2025 (Molson Coors, 2016).

Finally, one of the most interesting developments has been a rise in the development of low/NABs in the craft sector. Brewdog were one of the first to produce a 0.5% ABV hoppy ale named Nanny State, with Adnams producing a 0.5% ABV Ghostship to stand up against their 4.5% ABV equivalent. The opening of breweries that solely focus on the production of NABs such as Big Drop Brewing Co. and Nirvana Brewery have been key to driving even more development in this sector, with large product ranges and fascinating innovation including stouts and sours (Euromonitor, 2017c, Mintel, 2019b).

1.3 Production Methods

The production of NABs can be divided into two main categories: biological and physical methods. Biological methods focus on limiting ethanol production early on in the process, whilst physical methods remove ethanol post brewing. Numerous reviews have extensively detailed the different techniques (Branyik et al., 2012, Güzel et al., 2020, Liguori et al., 2018b, Montanari et al., 2009, Sohrabvandi et al., 2010), therefore a brief overview will be given in further sections.

1.3.1 Biological

Biological methods to produce NAB include; altered mashing, limited fermentation through batch or continuous processing, arrested fermentation and the use of special yeasts. Although none of these are new methods, they are starting to receive increased interest, especially in the craft sector, due to low investment costs compared to the purchasing of expensive equipment for continuous fermentation or physical methods (Bellut and Arendt, 2019).

1.3.1.1 Altered Mashing

Different strategies can be followed to produce a wort with low carbohydrate content, which include; high temperature mashing (75-80°C) to inactivate β-amylase, cold water malt extraction (<60°C) so that starch is unable to gelatinize, remashing spent grain which has lower fermentable sugars, and using new barley varieties which are β-amylase deficient (Branyik et al., 2012, Sohrabvandi et al., 2010). These are not new techniques, yet a recent study by Ivanov et al. (2016) explored these in detail by observing the effect of altered mashing times and temperatures (50 and 77°C) to obtain a wort with a reduced content of fermentable sugars, whilst comparing results

with a control mash. Although many physical parameters were measured, the influence of these parameters on the overall sensory characteristics was not recorded (Ivanov et al., 2016).

Beers produced through this method have commonly been reported to have increased sweetness and worty-off flavours, considered to be negative sensory characteristics, resulting from higher amounts of unfermented sugars and aldehydes respectively (Liguori et al., 2018b). As such, Branyik et al. (2012) suggested that using this method by itself does not yield a desirable NAB, and therefore it should be combined with other processing procedures such as wort boiling and acidification to lower aldehyde levels, and limited fermentation to limit ethanol production.

1.3.1.2 Limited Fermentation

Limited fermentation can be divided into batch or continuous processes, but both of these techniques rely on reducing yeast activity to limit ethanol production.

1.3.1.2.1 Batch Processing

Limited fermentation reduces the activity of the yeast, by lowering the temperature to around 0-4°C and extending fermentation times to 24-48 hours through cold contact processing (CCP) (Sohrabvandi et al., 2010). Under these conditions ethanol production is slow but formation of higher alcohols and esters is increased compared to other production methods (Branyik et al., 2012). Aldehydes responsible for worty off-flavours in beer, such as 3-methylbutanal, 2-methylbutanal and 3-methylpropionaldehyde, however are not reduced (Perpete and Collin, 1999). Improvements to this technique have

been suggested, which include using genetically modified yeasts which are less susceptible to higher temperatures to reduce worty aldehydes, as well as using polyvinylpolypyrrolidone (PVPP) after wort cooling to further reduce levels of aldehydes binding to polyphenols in the wort (Perpete and Collin, 1999, Perpete and Collin, 2000). However, no studies to the authors' knowledge have reported the impact of this technique on the sensory properties of these beers.

1.3.1.2.2 Continuous Processing

Continuous production of NAB is performed through the use of cold contact processing (CCP) and immobilised yeast using a bioreactor (Montanari et al., 2009). This also occurs at a reduced temperature, for longer fermentation times (Güzel et al., 2020). Immobolisation is achieved through four techniques which include; attachment of yeast cells to a solid support, entrapment of yeast cells within a porous matrix and containment of yeast cells behind either a microporous membrane filter or in microcapsules (Montanari et al., 2009). The objective of these procedures is to reduce negative aldehyde flavours produced by yeast. The lower temperature and anaerobic conditions means that the yeast is at an optimal steady state, thus growth is suppressed, limiting ethanol production and the oxidation of wort lipids to aldehydes (Verbelen et al., 2006). The Bavaria Brewery in the Netherlands have used this continuous technique to develop a packed bed immobolised yeast bioreactor to produce 150,000hL alcohol free beer per year (Van Dieren, 1995). Again, no studies to the authors' knowledge have looked at the impact of this technique on the sensory properties of NAB.

1.3.1.3 Stopped/Arrested Fermentation

Stopped or arrested fermentation occurs when yeast is removed before attenuation. This can be achieved through either temperature inactivation of the yeast, where temperature is reduced to 0°C or the product is pasteurised at high temperatures, or by removing yeast from fermenting wort by centrifugation or filtration (Branyik et al., 2012, Montanari et al., 2009). These processes require wort with a low concentration of fermentable sugars, with fermentation conducted at lower temperatures (2-3°C) for 150-200hours (Montanari et al., 2009). To prevent yeast from reproducing during this time, wort is not aerated, but this means that positive flavours such as higher alcohols and esters, normally produced during fermentation, are not formed. Instead, high sulfur compound content is usually found within these beers as they are not completely evaporated during wort boiling (Montanari et al., 2009). Improvements to the overall sensory characteristics of beers produced by this method have been found, including; altering malt composition to mask worty off flavours (Narziß et al., 1992), diluting wort after boiling to enhance ester and higher alcohol production (Narziß et al., 1992), acidifying wort to suppress worty character (Narziß et al., 1992, Schur and Sauer, 1990) and cold stripping wort with CO_2 or N_2 to remove undesirable sulfur compounds (Montanari et al., 2009). None of these studies, however used a trained sensory panel to quantify the changes in sensorial profiles.

1.3.1.4 Special Yeasts

The use of special yeasts in non-alcoholic brewing has gained momentum in recent years and can be seen as a trending topic, with several papers published showing advancements in this sector (Bellut and Arendt,

2019, Güzel et al., 2020). This can be divided into two different methods; selecting special yeast strains which are unable to ferment wort sugars, or using genetically modified yeasts (Liguori et al., 2018b).

1.3.1.4.1 Special Strains

The theory of using special strains means yeast is selected due to its limited ability to ferment maltose, the major fermentable sugar in wort (Branyik et al., 2012). Conversion of glucose, fructose and sucrose through fermentation leaves a beer with lower alcohol content, but a higher amount of glycerol, sugar alcohols and residual extract (Sohrabvandi et al., 2010). By-products which develop aroma can also be produced (Basso et al., 2016). A comprehensive review on the use of non-conventional yeast in producing NABLABs was recently published by Bellut and Arendt (2019) and will be discussed further.

Saccharomycdes ludwigii is the most commonly known NAB yeast strain, which has also been used in commercial production (Capece et al., 2018, Michel et al., 2016, Saerens and Hendrik Swiegers, 2014). This yeast is unable to ferment maltose and maltotriose and is slow to attenuate, therefore taking longer to ferment. There is however, no special need for continuous monitoring (Güzel et al., 2020). In terms of finished product quality, *Saccharomycdes ludwigii* has been found to produce beers with lower amounts of esters and higher alcohols (Liu et al., 2011) and diacetyl levels below threshold (De Francesco et al., 2015b), resulting in a weak aroma and sweet taste (Liu et al., 2011) as well as immature flavours with a low acceptance rate (Mortazavian et al., 2014). It was also found to produce a high level of decanoic acid, which has a cheesy/rancid off-flavour in beer (Saerens and Hendrik Swiegers, 2014).

Pichia kluyveri strains are gaining increased interest in usage by breweries, as a commercial strain is now available to purchase (Chr. Hansen A/S, Denmark). *Pichia* strains have a limited ability to ferment glucose. In recent studies a NAB with 0.1-0.2% ABV was produced and found to have a flavour profile similar to that of a standard alcohol beer. Similar levels of higher alcohols and esters and lower levels of diacetyl were found, as well as taste assessments showing a typical beer-like flavour, which was preferred over commercial NAB (Saerens and Hendrik Swiegers, 2014).

Zygosaccharomyces strains such as *Z. rouxii* and *Z. kombuchaensis* have also been utilised in previous studies, with these strains consuming ethanol under anaerobic conditions (Narziss et al., 1992; Sohrabvandi et al., 2009; Liu et al., 2011; De Francesco et al., 2015b). These strains however produce more off-flavour compounds such as diacetyl (Bellut et al., 2018, De Francesco et al., 2015b), have higher ethanol concentrations (0.93% ABV), produce lower levels of esters and higher alcohols (Bellut et al., 2018) and have low sensory acceptance (Mortazavian et al., 2014) presumably due to wort-like and honey-like sensory descriptors (Bellut et al., 2018), which are generally undesirable.

Certain strains of *Torulaspora delbrueckii* are unable to utilize maltose, making them ideal for low alcohol beer production. This yeast has been found in the environment and in the spontaneous fermentations of beer and wine (Güzel et al., 2020). Research using this strain has produced beers with diacetyl concentrations below threshold (Bellut et al., 2018, Michel et al., 2016), yet low ester and higher alcohol levels were reported (Bellut et al., 2018). Sensory analysis reported beers to have honey and pear-like characteristics (Michel et

al., 2016) and estery, fruity and citric sensory profiles, with a full-bodied mouthfeel (Canonico et al., 2016), which are desirable traits. One study however, reported less desirable sensory characteristics such as wort-like and bread-like, with products not discriminated from those made with *S. ludwigii* (Bellut et al., 2018).

Other non-Saccharomyces yeasts such as *Cyberlindnera mrakii*, have shown positive results to produce a low alcohol beer with a fruity aroma (Liu and Quek, 2016). *Mrakia gelida* produced beers with significantly increased fruity sensory profiles compared to *S. ludwigii* with descriptors such as apricot, grape and litchi, whilst also enhancing body (De Francesco et al., 2018). *Candida zemplinina* was also found to be a useful yeast when brewing with adjuncts, but no sensory study was conducted (Estela-Escalante et al., 2016).

1.3.1.4.2 Genetically Modified

The use of genetically modified (GM) yeasts has found hindrances due to negative consumer perception, therefore breweries have not been known to use these commercially (Güzel et al., 2020, Liguori et al., 2018b). Nevertheless, this area is receiving increased interest, with recent improvements in genomic engineering with technologies such as CRISPR/Cas. Over time it is believed more of these GM yeasts will be used commercially (Branyik et al., 2012, Güzel et al., 2020, Löbs et al., 2017, Stovicek et al., 2017). The principles of this process are that yeast can be modified by either gene deletion in the citric acid cycle or random mutation to produce NAB. One example is alcohol dehydrogenase (ADH)-negative yeasts, named as they are unable to produce ADH, an enzyme used for the important last step of fermentation converting acetaldehyde to ethanol. Other examples include yeast

mutants lacking in 2-ketoglutarate dehydrogenase (KGD) and fumarase (FUM) activity (Branyik et al., 2012). This process has been used before, however high acetaldehyde, diacetyl and acetoin content at the end of the process gave an unpleasant off-flavour (Dziondziak and Holsten Brauerei, 1989, Nevoigt et al., 2002, Selecky and Smogrovicova, 2007). Other mutants have also been used to produce beers with ethanol content <0.5% ABV and these were found to produce more lactic acid, therefore reducing risk of contamination and worty off-flavours (Selecky and Smogrovicova, 2007). In addition, these methods also showed increased glycerol levels, which have been previously shown to improve the mouthfeel and body of beer (Branyik et al., 2012). Theoretically, GM yeasts show a lot of potential for low/no alcohol beers with acceptable sensory characteristics but little published data is currently available.

1.3.2 Physical

Recent studies have produced detailed reviews on the physical methods of creating NABs (Mangindaan et al., 2018, Müller et al., 2017) with further grouping into thermal and membrane based processes.

1.3.2.1 Thermal

The oldest and most well-known thermal dealcoholisation technique is distillation, however this causes severe losses of important volatile components due to thermal stress, increasing the colour and caramelising naturally occurring sugars (Branyik et al., 2012, Montanari et al., 2009). Therefore newer production techniques such as rectification and evaporation were developed using heating with steam or liquid to remove ethanol due to its volatility. Improvements to these methods have occurred over the years to reduce volatile losses and lower extensive amounts of energy used (Blanco et

al., 2016). Vacuum distillation and spinning cone column (SSC) are known to be used on an industrial scale, with reduced thermal stress (Zufall and Wackerbauer, 2000b). These methods will therefore be discussed in further detail in upcoming sections.

1.3.2.1.1 Rectification

Rectification, or vacuum distillation builds upon the theory of distillation, yet this is performed under vacuum (4 to 20kPa) to reduce boiling temperature and ensure thermal stresses to flavour and colour are reduced (Branyik et al., 2012). This technique occurs by firstly degassing the beer, and then preheating to mild temperatures (30-60°C) in a plate heat exchanger, to dealcoholise using a packed-bed rectifying column (Branyik et al., 2012, Zufall and Wackerbauer, 2000b). Beer contacts rising vapours in counter flow, bringing selective separation of ethanol from the product. The dealcoholized beer is then passed through a cooler. Ethanol vapours are collected and concentrated in the rectification section and aroma components can be reintroduced into the beer (Montanari et al., 2009). Unfortunately no studies to date have reported the effect of this technique on sensorial properties of beer, however this technique is being used by craft-brewers to produce NAB, showing promising results (personal communications).

1.3.2.1.2 Thin Film Evaporators

Thin film evaporators have been engineered as an improvement to other techniques by reducing the contact time of ethanol on the evaporating system. This is achieved by flowing the beer as a thin film with a large surface area down the device, either mechanically, with products such as the Centritherm or

spinning cone column (SCC) systems (Flavourtech, Griffith, Australia) or gravimetrically with products such as the falling film evaporator.

Mechanical systems such as the Centritherm are designed similarly to a plate centrifuge, which is operated under vacuum at temperatures between 35-60°C, using steam as the heating medium. Beer enters the evaporator and is spread as a thin layer (approximately 0.1mm) over the heating medium, due to centrifugal force (Branyik et al., 2012). The dealcoholised beer is collected as a concentrated product at the outer edge of the cones and exits the evaporator. Volatiles removed through this technique rise up through the centre of the system as a vapour and are condensed externally. SCC, another mechanical technique, contains alternating fixed and rotating cones, with the fixed cones attached to the inside wall of the column and the others attached to a rotating shaft (300-500rpm). When beer is entered into the system from the top of the column, gravity pulls beer downwards, with the product dropping onto the first rotating cone, spinning the beer into a thin film. This continues down to the bottom of the column. Counter-currently, steam produced from water is used as the stripping medium, which is fed from the bottom of the column and rises upwards, passing over the surface of the thin film and collecting ethanol and other volatiles from the beer. This vapour is then collected at the top of the column where it condenses, capturing the volatiles in a concentrated liquid form (Branyik et al., 2012, Güzel et al., 2020). Previous studies dealcoholizing beer using these techniques employed a combination of SCC to dealcoholize beer down to 0.02% vol, yet a significant loss of important volatile compounds occurred (Catarino and Mendes, 2011b). Pervaporation (discussed in section 1.3.2.2.3) was then used to counteract these losses, by extracting the volatile

compounds from the permeate. This flavour cocktail, as well as original and dealcoholized beers were then blended back to an ethanol concentration of 0.45% vol. A trained sensory panel stated that this significantly improved the flavour profile of the beer (Catarino and Mendes, 2011b).

Falling film evaporators use gravity as the overall force to dealcoholize beers. Firstly beer is preheated to evaporation temperature (30-60°C) with a saturated steam under vacuum and enters the column, flowing downwards (Branyik et al., 2012). This occurs through a distributor device, which ensures that an even liquid film is distributed on the inner walls of the tubes. The beer is partially evaporated and is then passed into a condenser. Beer is only in the evaporator for a few seconds, making it a very efficient technique (Montanari et al., 2009). Falling film evaporation was previously studied, with research showing potential to dealcoholize a beer to 0.51% vol (Zufall and Wackerbauer, 2000b). Even through changing certain parameters, a loss of 95% higher alcohols, 99% total esters and 48% fatty acids was shown, therefore producing a product that was significantly different from the original beer (Zufall and Wackerbauer, 2000b). Unfortunately no sensory study was conducted to understand the changes in sensorial profile using this technique.

1.3.2.2 Membrane

Membrane processes include; dialysis, osmotic distillation (OD), pervaporation, reverse osmosis (RO) and nanofiltration (NF). These are some of the most promising techniques for NAB production, due to low energy consumption, low temperature operation, high separation efficiency and low cost. Mangindaan et al. (2018) showed that almost 50% of publications in recent years have focused on the use of these methods to produce non-alcoholic

beverages. In this section the theory of dealcoholisation in the most common membrane processes will be discussed, as well as outcomes from publications using these techniques. A comparison of relative losses of volatiles for each membrane based process within beer dealcoholisation are also shown in Table 1.2.

1.3.2.2.1 Dialysis

Dialysis is one of the earliest applications of membrane-based dealcoholisation, which uses the same principle as the technique most commonly recognised in medical fields (Güzel et al., 2020). The process occurs due to a transmembrane concentration difference, which pushes beer over a semipermeable membrane. An alcohol-free dialysate (normally water) flows counter-currently to the beer on the other side of the membrane. The membrane acts as a molecular sieve permeable to ethanol, which diffuses through from an area of high concentration (beer) into an area of low concentration (dialysate). Although this method relies upon diffusion, pressure must also be applied to minimise losses of CO_2. Dialysis membranes are normally composed of cellulose acetate, polyamide, polysulphone and polyethersulphone, but the most popular and commercially available are cellulose (Cuprophane). These membranes are usually arranged in bundles of hollow fibres, called modules (Branyik et al., 2012). Dialysis has been reported in several studies dealcoholizing beer (Leskošek et al., 1995, Leskošek and Mitrović, 1994, Petkovska et al., 1997). Zufall and Wackerbauer (2000a) found that nearly all esters and higher alcohols and up to 50% of short-chain fatty acids were removed, yet no sensory research was conducted to understand the effect on flavour profile. Therefore although dialysis is one of the oldest and

most established techniques, which has been around for almost 30 years, research is now shifting to newer dealcoholisation technologies.

Table 1.2: *Percentage change of selected properties of NABs calculated from original input beer and NAB obtained by different membrane dealcoholisation methods*

Changes to Important Brewing Parameters	Dialysis (Zufall and Wackerbauer, 2000a)			Osmotic Distillation (Liguori et al., 2015)			Pervaporation (Pollock, 1990)			Reverse Osmosis (Kavanagh et al., 1991)		
	Start	End	**% Change**	Start	End	**% Change**	Start	End	**% Change**	Start	End	**% Change**
Ethanol (% ABV)	4.8	0.5	**-90**	5.0	0.5	**-90**	4.6	0.6	**-87**	4.9	0.4	**-92**
Colour (EBC)	7.3	7.5	**+3**	7.6	8.4	**+11**	7.3	10.8	**+32**	N/A	N/A	**N/A**
pH	4.6	4.7	**+3**	4.1	4.2	**+2**	4.7	4.7	**0**	N/A	N/A	**N/A**
Bitterness (EBC)	30.7	29.7	**-3**	16.0	13.0	**-19**	22	32	**+31**	24.6	12.3	**-50**
Total Higher Alcohols (mg/L)	69.9	2.7	**-96**	54.2	2.5	**-95**	53.9	2.1	**-96**	148.0	27.9	**-81**
Total Esters (mg/L)	14.3	<0.1	**-99**	14.9	0.2	**-99**	15.5	0.0	**-100**	17.6	2.0	**-89**
Total Fatty Acids (mg/L)	8.8	4.3	**-51**	N/A	N/A	**N/A**	N/A	N/A	**N/A**	7.9	0.9	**-89**

1.3.2.2.2 Osmotic Distillation

Osmotic distillation (OD) (also known as membrane contactor, isothermal membrane distillation and evaporative pertraction), separates volatile components from a liquid mixture using a microporous hydrophobic membrane. These membranes are normally polypropylene based (Mangindaan et al., 2018). In the first step of this process, evaporation occurs. Ethanol in the beer evaporates at the pores of the beer side, with this ethanol vapour diffusing through the pores of the hydrophobic membrane and condensing in the stripping solution on the permeate side of the membrane (Müller et al., 2017). No pressure is applied in this technique, as the driving force for ethanol transport is the difference in vapour pressure between the beer and the stripping solution. Previous research has used this technique successfully in the partial dealcoholisation of wine (Diban et al., 2008, Gambuti et al., 2011, Liguori et al., 2013b, Liguori et al., 2013a, Lisanti et al., 2013, Varavuth et al., 2009), as well as recent research into NAB production conducted in a pilot plant setting (De Francesco et al., 2015a, Ejikeme et al., 2013, Liguori et al., 2015, Liguori et al., 2018a, Russo et al., 2013). De Francesco et al. (2015a) used OD to remove alcohol from different beers, one of which had an enhanced starting volatile profile to counteract the high losses found in membrane dealcoholisation techniques. A decrease in ethanol concentration by 81% was found (final ethanol concentration 0.9% vol), yet with this decrease a significant loss to below sensory threshold of higher alcohols (83%), esters (84%) and aldehydes (44%) occurred. This had an impact on the sensory profile, with reduced estery flavours as well as sweetness, body and lingering aftertaste. The authors concluded that a beer with a characteristic estery flavour

profile should not be used for OD, but instead manufacturers should focus on a more malty character as this attribute was unchanged during dealcoholisation (De Francesco et al., 2015a). Other studies looked at the influence of different operating techniques, such as temperature, stirring speed and membrane pore size (Ejikeme et al., 2013), as well as the use of different stripping solutions (water and alcoholic solutions) (Russo et al., 2013). More research was also conducted to reduce the environmental impact of this technique by using recycled stripper solutions from preliminary dealcoholisation trials (Liguori et al., 2015). Finally, the use of carbonated stripper solutions was explored (Liguori et al., 2018a), which was found to reduce losses of higher alcohols to 68%, esters to 71% and aldehydes to 41% (although this beer had a final ethanol concentration of 1.1% vol). Sensory descriptors showed that the original beer was characterised by body, fruity/esters, fruity/citrus, malty and alcohol descriptors, whereas dealcoholized beers were characterised by burnt, astringent and cereal (Liguori et al., 2018a).

1.3.2.2.3 Pervaporation

Pervaporation is commonly used in combination with distillation to dehydrate ethanol from water to >98% purity (Mangindaan et al., 2018). Here, however pervaporation is used to dealcoholize beer, with the theory of pervaporation occurring through evaporation and permeation over a hydrophobic membrane (Wenten et al., 2017). Separation occurs during the transition from a liquid to a vapour, due to a difference in partial pressures as well as selectivity of the membrane (Pollock, 1990). Beer is contacted onto the membrane at around 50°C, with no pressure applied (Mangindaan et al., 2018). The components in beer interact with the polymer membrane, which is non-

porous and selectively permeable, and diffuse through a concentration gradient from the permeate side. Selectivity of the membrane is due to diffusion coefficients as well as solubility (Müller et al., 2017). Once the permeate has passed through the membrane, it passes into the gas phase and is deposited onto a condenser. The permeate stream can then be collected for further use (Feng and Huang, 1997).

The disadvantages of using this method separately are clear, as this process can be time consuming and shows large losses of volatile flavour compounds when dealcoholizing down to only 2.6% vol (del Olmo et al., 2014, Pollock, 1990). Combining this method with other techniques has shown promising results, with the reuse of the waste permeate stream in blending. Therefore pervaporation has mainly been reported for producing NAB in the field of aroma recovery (see section 1.3.2.1.2). del Olmo et al. (2014) also used a similar technique to that of Catarino and Mendes (2011b) to recover higher alcohols and esters from two different dealcoholised beers, which were then blended to produce a beer with a sensory profile deemed acceptable by trained sensory panellists. Unfortunately these authors used unsuitable sensory analysis techniques, as they asked trained sensory panellists to assess their preference of the samples, which is not sensory best practice.

1.3.2.2.4 Reverse Osmosis

Reverse osmosis (RO) has been used for many applications including water and wastewater treatment, fruit juice concentration and milk separation (Wenten and Khoiruddin, 2016). It has also been shown as a promising technique to dealcoholize fermented beverages such as beer, wine and cider (Alcantara et al., 2016, Catarino et al., 2006, Catarino et al., 2007, Gil et al.,

2013, Lopez et al., 2002). Pressurised beer (20-80 bar) is passed through a semi-permeable membrane, with molecules such as ethanol and water removed into the permeate, whilst larger flavour molecules are retained in the product. CO_2 is lost during the process and so the final product must be carbonated after dealcoholisation (Hodenberg, 1991). As there are large water losses during processing, water needs to be added back in via diafiltration, which can be described as continuous (adding water back in during processing at different time points) or discontinuous (by diluting the product at the beginning or rediluting at the end) (Branyik et al., 2012). Membranes are selected due to their characteristics, including: high selectivity of ethanol, low selectivity of important beer components such as volatile aroma and flavour compounds, resistant to temperature, chemicals, fouling and cleaning/disinfecting agents, as well as being inexpensive (Branyik et al., 2012, Pilipovik and Riverol, 2005). Membranes can be made from either cellulose acetate, polyamide or polyimide on polyester, polysulphone, or fibreglass support structures (Branyik et al., 2012). These are then normally placed in geometric arrangement modules, which can include planar, tubular or spiral-wound (Light et al., 1986). Previous research conducted using RO included research on different operating parameters with homemade alcoholic beverages and beer (Catarino et al., 2006, Pilipovik and Riverol, 2005), as well as on different RO membrane materials with both cider (Lopez et al., 2002) and beer (Catarino et al., 2007). Catarino et al. (2007) found that cellulose acetate membranes were the most promising, as they exhibited the highest permeate flux and lowest ethanol rejection. Similar results were found by Lopez et al. (2002), whilst also studying the use of different operating modes (continuous and discontinuous diafiltration). In more

recent studies RO was used to dealcoholize a stout style beer, whilst also analysing the effects of operating temperature and initial dilution (Alcantara et al., 2016). Unfortunately this research failed to assess the impact on sensorial characteristics.

1.3.2.2.5 Nanofiltration

Nanofiltration (NF) is a pressure-driven technique, similar to RO, which uses pressure to pass beer through a semipermeable membrane. Membranes are in-between ultrafiltration (UF) and RO in terms of characteristics, thus NF has tighter pores than UF and exhibits better rejection for smaller molecules, but looser pores compared to RO (Mangindaan et al., 2018). NF has been used predominantly in the dealcoholisation of wine, with promising results (Catarino and Mendes, 2011a, Labanda et al., 2009) yet few studies have reported use in the dealcoholisation of beer. Mangindaan et al. (2018) reported that this technique could be a promising alternative to RO for beverage dealcoholisation.

1.3.3 Additional Techniques to Improve Quality

To improve the sensorial quality of NAB, many breweries use different pre and post-treatment methods, blending techniques and additives (Branyik et al., 2012, Müller et al., 2017). Pre-treatment ideas include brewing at a higher gravity to obtain a more aromatic beer further down the line, using stepped mashing procedures to inactivate B-amylase, changing the final fermentation degree and improving mouthfeel, as well as using non-malted barley and non-fermentable maltose (Müller et al., 2017). Post-treatment suggestions include; blending with original beer, aromatic beer or krausen (Müller et al., 2017), addition of fresh yeast followed by maturation (Branyik et al., 2012), late or

dry hopping (Forster and Gahr, 2011), addition of hop extracts post production and using aroma concentrate from aroma recovery, obtained from industrial rectification plants (Liguori et al., 2016). Additives can also be used post-treatment and these may include; saccharin, ascorbic acid, lactic acid, citric acid, potassium metabisulphite, caramel colouring, glucose-fructose syrup and dextrins (Branyik et al., 2012).

Table 1.3 summarises the key advantages and disadvantages found for each production method. Overall, the majority of research to date has investigated the impact of ethanol reduction and removal methods on the composition of the final beer matrix, measured using instrumental techniques, and have lacked robust sensory research. Therefore the real potential for these methods is currently somewhat unclear. Ethanol plays an important role in beer, not just influencing its sensory properties, but also in the physicochemical interactions within the beer and the by-products of its production via fermentation. Therefore, in order to fully understand the impact of its removal, the physicochemical and sensory properties of ethanol must be understood.

***Table 1.3**: Summary of advantages and disadvantages of all non-alcoholic beer production techniques*

	Production Methods		Advantages	Disadvantages
Biological	Altered Mashing		➢ Traditional brewing equipment can be used, so no large investment in specialist equipment is needed[1]	➢ Risk of microbial contamination[1] ➢ Increased sweetness and worty off-flavours, considered to be a negative sensory characteristic[1] ➢ Higher amounts of aldehydes and unfermented sugars[1] ➢ Has to be combined with other methods to produce a more desirable beer[2]
	Limited Fermentation	Batch Processing	➢ Economical[3] ➢ Can produce beers <0.1% ABV[3]	➢ Constant analytical controls needed, with checks needed every 8 hours[3] ➢ High yeast cell starting concentrations[1]
		Continuous Processing	➢ Lower production costs[3] ➢ Rapid start-up phase[3] ➢ Improved utilisation of raw materials[3]	➢ Investment in specialist equipment[2]
	Stopped/Arrested Fermentation		➢ Traditional brewing equipment can be used, so no large investment in specialist equipment is needed[2]	➢ Difficult to achieve low ethanol levels[2] ➢ Increased sweetness and worty off-flavours, due to non-reduced aldehydes[24] ➢ Constant analytical controls needed[24]

	Special Yeasts	Special Strains	➢ Traditional brewing equipment can be used, so no large investment in specialist equipment is needed[5]	➢ Risk of microbial contamination, so higher standards of cleanliness and microbiological control should be followed[6]
		Genetically Modified	➢ Increased glycerol levels, improving body[2]	➢ Negative consumer perception[1]
Physical	Thermal	Rectification	➢ Ethanol can be removed from beer completely[2] ➢ Ethanol recovered from separation can be resold commercially[2] ➢ Operation automatic and continuous[2]	➢ Investment in specialist equipment[2] ➢ Consume high amounts of energy[2] ➢ High thermal damage to products[2] ➢ Finished product has to be diluted and carbonated[3]
		Thin Film Evaporators	➢ Short contact time[3] ➢ Low thermal damage to product[3] ➢ Falling film systems – no oxygen transfer can occur[9]	➢ Investment in specialist equipment[2] ➢ Severe losses of volatile compounds found[7] ➢ Bland and flavourless product produced[7] ➢ Energy extensive process[7] ➢ Finished product has to be diluted and carbonated[8] ➢ Mechanical systems - potential for oxygen to penetrate the system[9]
	Membrane	Dialysis	➢ Occurs at low temperatures, so no thermal stress to product[1] ➢ Finished product does not need to be carbonated[9]	➢ Investment in specialist equipment[2] ➢ Large losses of volatile compounds found[9] ➢ Reduction below 0.5% ABV not possible[9]

		Osmotic Distillation	➢ Can be operated at room temperature, so no thermal stress to product[10] ➢ Lower pressures needed compared to other methods[10] ➢ Water stripping solution can be reused[11]	➢ Investment in specialist equipment[2] ➢ High membrane fouling[12] ➢ High volumes of stripping solution needed[12] ➢ High operating costs[12]
		Pervaporation	➢ Can be used in aroma recovery with product being blended back into dealcoholized beer[14]	➢ Investment in specialist equipment[2] ➢ Time consuming[13] ➢ Large losses of volatile compounds[13]
		Reverse Osmosis	➢ Occurs at low temperatures, so no thermal stress to product[8] ➢ Reduced energy consumption[8] ➢ Produces beers with lowest volatile reduction in comparison to other methods[16]	➢ Investment in specialist equipment[2] ➢ Not economically feasible to produce beer below 0.45% ABV[15]
		Nanofiltration	➢ Occurs at low temperatures, so no thermal stress to product[7] ➢ Reduced energy consumption[7] ➢ Shorter time to dealcoholize compared to RO[7] ➢ Successfully used to dealcoholize wine[17]	➢ Investment in specialist equipment[2] ➢ Not used before in dealcoholisation of beer[7] ➢ Increased losses of volatile compounds compared to RO[17]

[1](Liguori et al., 2018b), [2](Branyik et al., 2012), [3](Montanari et al., 2009), [4](Perpete and Collin, 1999), [5](Bellut and Arendt, 2019), [6](Muller, 1990), [7](Mangindaan et al., 2018), [8](Müller et al., 2017), [9](Zufall and Wackerbauer, 2000a), [10](Lawson and Lloyd, 1997), [11](De Francesco et al., 2015a), [12](Liguori et al., 2015), [13](del Olmo et al., 2014), [14](Catarino and Mendes, 2011b), [15](Pilipovik and Riverol, 2005), [16](Catarino et al., 2007), [17](Catarino and Mendes, 2011a)

1.4 Physicochemical and Sensory Effects of Ethanol

1.4.1 Analytical Techniques for Measuring Volatiles in Ethanolic Solutions

Many methods can be used to identify and measure the release of volatile compounds from a food or drink matrix. The most widely used technique is gas chromatography mass spectrometry (GC-MS), using fused silica capillary columns and a single quadrupole mass spectrometer, with helium as the carrier gas (mobile phase) (Elmore, 2015). Samples are injected onto a heated column, with compounds separated at different retention times due to their volatility. Compounds are bombarded with electrons, partially fragmenting and producing a characteristic library-searchable mass spectra (Elmore, 2015). Although using a library is a useful technique in identifying compounds, they must then be confirmed using pure analytical standards. One drawback of GC-MS however, is that it is too slow for real-time analysis, which can be useful for measuring changes during consumption.

Techniques that can be used to capture real-time analysis of volatile partitioning are Atmospheric Pressure Chemical Ionisation-Mass Spectrometry (APCI-MS) or Proton Transfer Reaction- Mass Spectrometry (PTR-MS) which are both soft ionisation methods, meaning compounds are not fragmented to a high degree (Taylor et al., 2000). APCI-MS occurs by drawing the sample through a heated fused silica capillary inlet. An initial reactant ion is formed, which can transfer its charge to any molecule with a higher proton affinity at atmospheric pressure. The ionised compounds are protonated by the transfer of charge from the reactant ion, with the resultant ions sampled into a MS for quantification (Taylor et al., 2000). One issue with measuring ethanolic

samples above 4% v/v is that significance in the ionisation behaviour of aroma compounds has been found, so results were inconsistent (Aznar et al., 2004). To tackle this, Aznar et al. (2004) developed a technique in which ethanol is added as the proton transfer reagent ion at the source. In PTR-MS, the volatiles are ionised due to their smaller proton affinity compared to water, via a proton transfer reaction from H3O+. This occurs in a buffer gas, usually air, which flows into a drift tube. A mass analyser detects the ionic products at the end of this drift tube (Blake et al., 2009).

1.4.2 Physicochemical Properties of Aroma Compounds

Aroma compounds are found at very low concentrations in beer, which are in the infinite dilution range of lower than 10^{-4} (Athès et al., 2004). The availability of these volatile aroma compounds in the headspace can be governed by not only their concentration within the food or drink matrix, but also their volatility, chemical reactivity, vapour pressure, solubility, partition coefficient and hydrophobicity (Fisk, 2015). This means that, although a compound may be present at a high concentration within a beer, interactions between the compounds and other matrix components can determine the intensity of flavour release from the product (Fisk, 2015). These physicochemical properties are important factors in understanding the functionality of compounds, and will be further discussed.

The partition coefficient is the ratio of a volatile compound concentration between the gas and liquid phases at equilibrium, which is shown in equation 1.1, where K_{aw} is the partition coefficient, C_a is the concentration of solutes in the air phase and C_w in the water phase.

$$K_{aw} = \frac{C_a}{C_w} \qquad \text{(equation 1.1)}$$

This can also be expressed as a function of Henry's Law Constant, as shown in equation 1.2, where *H* is Henry's Law Constant, *P* is partial pressure of the volatile compound in the gas phase and C_L is the solubility of a gas in liquid phase.

$$H = \frac{P}{C_L} \qquad \text{(equation 1.2)}$$

The hydrophobicity of an aroma compound can also be defined as the solubility between water and lipid phases, with the result expressed as log*P*. The equation for calculating this is shown in equation 1.3, with C_O the concentration in the oil phase and C_w in the water phase. Hydrophilic molecules have a negative log*P*, whilst hydrophobic molecules have positive values (Taylor, 2002). Knowledge of all of these parameters is important to understand the availability of the compound and therefore release of flavours into the headspace.

$$logP = log\frac{C_o}{C_w} \qquad \text{(equation 1.3)}$$

1.4.2.1 Physicochemical Properties of Ethanol

Understanding the physicochemical properties of ethanol is key to understanding the influence it has on aroma perception. Ethanol is commonly used as a solvent due to its structure, with a high affinity to water, as well as a dual nature, meaning it is able to dissolve polar, ionic and non-polar substances (Athès et al., 2004, Taylor, 2002). Ethanol acts as a cosolvent with water in alcoholic beverages such as wine and beer, and can affect the solubility of volatile and non-volatile fractions dependent on its concentration in solution

(Tsachaki, 2006). Ethanol molecules have been found to be monodispersed at <15% ethanol v/v, yet when this is increased to 15-57% there is a progressive aggregation of ethanol molecules to form micelles, reducing the hydrophobic hydration of the alkyl chain. This continues until water loses its hydrogen bonded network completely and mixes into solution as a single molecule, which occurs at an ethanol content of >57%. The hydrogen bonded network of water is lost and the solution becomes water monodispersed in ethanol (D'Angelo et al., 1994a, D'Angelo et al., 1994b). Therefore, at concentrations appropriate for beer, ethanol remains monodispersed in water.

1.4.3 Effects of Ethanol on Aroma Delivery

The presence of ethanol within a solution such as beer, where concentrations are <20% v/v, have been found to decrease the concentration of volatiles in the headspace by up to 50% (Athès et al., 2004, Aznar et al., 2004, Fischer et al., 1996, Tsachaki, 2006). Studies suggested that the critical ethanol concentration for headspace partitioning was 17% v/v due to partitioning into ethanol clusters (Conner et al., 1998, Escalona et al., 1999), however more recent research suggests that this concentration may be lower at around 10% v/v (Athès et al., 2004, Aznar et al., 2004, Boelrijk et al., 2003, Tsachaki, 2006). It is believed that this effect occurs due to the presence of ethanol affecting the polarity of the product matrix, increasing the solubility of aroma compounds, thus reducing their concentration into the headspace (Aznar et al., 2004). In research relevant to beer, Clark et al. (2011b) discovered no difference in the release of ethyl acetate, isoamyl alcohol and phenylethyl alcohol in a beer-like matrix at 0 and 4.5% ABV. APCI-MS was used to measure static headspace at 4.5% ABV, and it is believed that this lack of

significance was due to the lower ethanol concentrations used (Clark et al., 2011b).

The above studies were conducted using static headspace techniques, however this fails to take into account other aspects normally found during consumption of food and drink, such as air sweeping, saliva mixing, mastication and temperature changes (Clark et al., 2011b). Dynamic methods have been developed to include some of these changes, with the earliest study of this by Elmore and Langley (1996) who studied the effect of headspace gas sweeping on four volatiles (maltol, vanillin, 2-heptanone and isoamyl acetate) in ethanol/water solutions of 5, 10, 20 and 40% ABV. Similar results were found to those using static headspace techniques, with volatile compounds decreasing as ethanol concentration increased. In another study, purge and trap and thermal desorption cold trap extraction was used by Perpete and Collin (2000) to measure aldehydes in beers with different ethanol concentrations. Increasing the ethanol concentration of the beer from 0 to 5% showed increased retention of aldehydes, such as 2-methylbutanal and 3-methylbutanal, which are responsible for the ‘worty’ off flavours in NAB. In another study, by Tsachaki et al. (2005), APCI-MS was used to understand dynamic release in ethanol/water solutions of 0 and 12% ABV. Increased ethanol concentration was found to increase the headspace concentration of volatiles, showing different conclusions compared to other studies (Aznar et al., 2004, Tsachaki, 2006). This phenomenon was explained through the Marangoni effect, in which ethanol lowers the surface tension of the solution, evaporating more easily. Flavour molecules move with this ethanol, therefore becoming more readily released into the headspace. Finally, Clark et al. (2011b) discovered a

significant difference on ethanol partitioning for ethyl acetate and isoamyl alcohol when using APCI-MS and measuring the short term decanting headspace (measured after opening the beer). Interactions between other beer components were found to affect volatile release, with hop acids increasing the release of ethyl acetate (Clark et al., 2011b). These studies show that ethanol has the capability to modify aroma release dependent on the concentration. However, in order to understand the impact of physiochemical interactions on perception, methods measuring volatile release in breath have been used.

The most similar technique to the real life dynamics of human consumption are in-vivo techniques. Boelrijk et al. (2003) compared static headspace results taken by GC-MS, dynamic results by APCI-MS-Nose using an artificial mouth and in vivo techniques using APCI-MS-Nose with panellists. The release of five aroma compounds (2-5-dimethylpyrazine, ethyl hexanoate, ethyl acetate, linalool and trans-2-nonenal) were measured in different ethanol/water solutions (2.5, 5, 10, 20 and 40% v/v). Compound release was discovered to be affected by ethanol concentration independently, with some compounds decreasing with ethanol concentration (ethyl hexanoate, ethyl acetate and trans-2-nonenal), whilst others were unaffected (linalool and 2-5-dimethyl pyrazine) (Boelrijk et al., 2003). This was also found in static headspace studies with model solutions (Aznar et al., 2004, Boothroyd et al., 2012). In a recent study, a different technique of intra-oral SPME sampling with real wines was used, but ethanol concentration was only significant in aroma release for some compounds (Muñoz-González et al., 2019). The authors concluded this was due to the physicochemical properties of the compounds, with the release of less polar compounds (such as ethyl octanoate

and ethyl decanoate) found to be lower in higher ethanol concentrations. It was believed this was due to their solubility in higher ethanol concentrations (Muñoz-González et al., 2019). Finally, Clark et al. (2011b), also used in-vivo techniques, by using APCI-MS with model beers, to measure the release of ethyl acetate, isoamyl alcohol and phenylethyl alcohol. Ethanol concentration was found to increase the in-breath release of volatiles, during the first breath after swallowing, due to a change in surface tension, solubility or the Marangoni effect (Clark et al., 2011b). More recent studies have also used aroma trapping techniques (Tenax), with model wines, showing similar results (Muñoz-González et al., 2014b).

Ethanol has been shown to have an impact on volatile release through static, dynamic and in-vivo measurements, but it is clear from previous research that there are conflicting results. The majority of these investigations have not reported the sensory impact of their findings. Therefore, in order to understand the impact of reducing ethanol content in beer (aside from the complexities introduced by production method) research needs to be conducted to understand the physicochemical interactions ethanol has with key aroma compounds at concentrations relevant to beer. Interactions with beer matrix components (such as proteins and carbohydrates) and the resulting impact on sensory perception should also be explored.

1.5 Sensory Perception of Ethanol

Ethanol is a multimodal stimulus which contributes to the aroma, taste, flavour and mouthfeel of all alcoholic beverages (Clark et al., 2011a). As this thesis aims to develop an understanding and improved sensory quality of low alcohol beer, the first challenge is to fully understand the sensorial complexity

of ethanol. A comprehensive review on the effects of ethanol perception in different alcoholic beverages including model alcohol solutions, wines, beers, ciders and spirits, was recently published by (Ickes and Cadwallader, 2017). Here the role of ethanol in concentrations relevant to beer (between 0-10% ABV) will be discussed, but other matrices will be mentioned where little or no beer-relevant research exists.

1.5.1 Aroma

Volatile molecules are able to enter the nasal cavity via two different pathways; orthonasally (through sniffing) or retronasally (through consumption). These aroma molecules interact with the olfactory neurons in the olfactory bulb, which are then sent along the olfactory nerve to the primary olfactory cortex. Multiple odorants can be detected by different olfactory receptors, resulting in detection of over 100,000 aroma compounds (Buck, 2004).

In early sensory studies the effect of dealcoholisation on cider, wine, sparkling wine, sherry and whiskey were interpreted, with results suggesting that the removal of ethanol increased fruitiness aroma perception (Williams, 1972, Williams and Rosser, 1981). In studies more relevant to beer, no significant difference was found in a model beer system with ethanol concentrations between 0 and 4.5% ABV for the aroma attributes of ‘sweaty/cheesy’ and ‘floral’ aromas (Clark et al., 2011a) suggesting that the impact of ethanol on aroma partitioning found in instrumental studies was not large enough to effect perception. Peltz and Shellhammer (2017) also discovered that ethanol concentration (5 and 10% ABV) had no effect on the aroma detection threshold of hop compounds in beer.

1.5.2 Taste

The oral sensation elicited by a response from chemoreceptors within the oral cavity is called taste. Humans are able to distinguish between five basic tastes which include; bitter, sweet, sour, salty and umami (Bachmanov and Beauchamp, 2007). The presence of ethanol has been found to have a significant impact on the tastes of sweet, bitter and to a lesser extent sour, with the threshold found in different studies ranging from 0.87-1.43% v/v (Mattes and DiMeglio, 2001, Nolden and Hayes, 2015), a key range in NAB. Previous research on the sensory properties of ethanol on taste will be further discussed.

1.5.2.1 Sweet

During wort preparation in the brewing process, starch is degraded by α-amylase and β-amylase to form sugars. Although during fermentation the yeast converts the sugars into ethanol, volatile flavour compounds and carbon dioxide, some sugar still remains and therefore all beers will have a sweet sensory aspect to them (Briggs et al., 2004).

Ethanol has been found to enhance sweetness perception in early studies by Martin and Pangborn (1970), due to an increased electrophysiological response of the chorda tympani nerve (a branch of the facial nerve originating from taste buds) in the presence of ethanol. Other studies have also confirmed that ethanol stimulates sweet-best fibres due to taste-taste mechanisms, as well as activating nerve fibres sensitive to sugar (Hellekant et al., 1997, Scinska et al., 2000). This was also found in more recent studies by Clark et al. (2011a) who found that ethanol increased sweetness perception in a beer matrix, relating this to the gustatory response of ethanol. Conversely, an increase in ethanol concentration up to 5% ABV has

been found to suppress the sweetness of different sugars, including sucrose and glucose (Hoopman et al., 1993).

1.5.2.2 Bitter

All beer has some level of bitterness due to the addition of hops during the brewing process (Briggs et al., 2004). These hops are thermally isomerised during the wort boiling stage of the brewing process to produce iso-α-acids, which are known for imparting bitterness (De Keukeleire, 2000). Bitter taste is recognised by around 25 receptors in humans when coupled with the G-protein gustducin (Upadhyaya et al., 2010). These receptors belong to the T2R family and can be activated by numerous bitter substances, the most commonly used and researched are quinine and caffeine. These substances have been researched extensively in understanding bitterness, however only recently have research articles explored the effect of hop acids within beer (Higgins et al., 2020, Higgins et al., 2021, Oladokun et al., 2016). Bitterness from hop acids in beer was found to be complex; a trained beer panel generated a total of thirteen bitterness descriptors ranging from 'harsh' to 'rounded' (Oladokun et al., 2016), with time intensity data showing that hop extracts follow a different temporal pathway compared to quinine and caffeine (Higgins et al., 2021).

Ethanol has been found to enhance bitterness, in the form of quinine, in a study by Martin and Pangborn (1970). Other research has also shown that the predominant taste of ethanol near threshold is bitter (Mattes and DiMeglio, 2001, Scinska et al., 2000), although this can change with concentration (Nolden and Hayes, 2015). This change was explained to be due to an additive effect on bitter sensation, which intensifies the flavour (Martin and Pangborn, 1970). Mattes and DiMeglio (2001) also found, using carbonated water, NAB

and standard beer rinses, that acute exposure to ethanol initially suppresses the bitterness of quinine, but augments its bitter aftertaste. Ethanol was also found to not significantly modify bitterness perception given by hop acids (Clark et al., 2011a).

1.5.2.3 Sour

All beer is acidic, with an average pH of 4.0±0.2 (Taylor, 1990). Acidity can be linked to sour taste, as this is stimulated by acid sensing ion-channels, which are depolarised by free protons. Common sour stimulants include acetic acid and citric acid (Roper, 2007).

Martin and Pangborn (1970) found that high concentrations of ethanol (24% ABV) suppressed sourness, in the form of citric acid. This was found in another study to be due to the decrease in physiological response of the chorda tympani nerve in the presence of another sour stimulus, acetic acid (Hellekant et al., 1997). Scinska et al. (2000) also found that a 10% ethanol solution was described to have a slightly sour component, although it was discussed afterwards that this could be linked to a mild burning sensation.

1.5.3 Mouthfeel

Langstaff et al. (1991) found nine attributes which were used to report differences in the mouthfeel of 30 commercial beers. The authors discussed that these could be grouped into three descriptors; carbonation (sting, bubble size, foam volume, total CO_2), fullness (viscosity and density) and afterfeel (oily moutcoat, astringency and stickiness). Only some of these attributes have been found to interact with ethanol and therefore these will be discussed in further sections.

1.5.3.1 Carbon Dioxide

Carbonation has been described to produce sensations of tingly/prickling and even sometimes pain response (Dessirier et al., 2000, Simons et al., 1999). This has been researched extensively in carbonated beverages, however little is known about the interaction between ethanol and carbon dioxide in alcoholic beverages. In a recent study using wine, Gawel et al. (2020) discovered a small interaction effect between dissolved carbon dioxide and ethanol, however this changed with the product matrix, with different changes in red and white wines (Gawel et al., 2020). In research relevant to beer, Clark et al. (2011a) found a complex interaction between carbonation and ethanol in a model beer system, influencing the perception of warming sensation. However, to the authors' knowledge no further studies have been conducted to understand this interaction within a real beer matrix.

1.5.3.2 Viscosity/Density/Body/Fullness

Viscosity and density can be measured analytically using viscometers and rheometers, but may also be used as sensory terms. Viscosity can be explained as the 'amount of force that must be applied to move the beverage around in the mouth' (Gawel et al., 2007) with density described as 'the weight of the liquid' (Pickering et al., 1998). The sensorial terms used to describe these physical attributes can also be described as 'body' or 'fullness' and are described as an 'overall impression of weight in the mouth' (Gawel et al., 2007). Confusion often lies between the use of these terms to help describe the mouthfeel of beverages. In the wine industry, 'body' is commonly used, however this term has been scrutinised over recent years as it is believed to be

an abstract sensory attribute with a multidimensional nature (Gawel et al., 2007).

In early sensory studies by Langstaff et al. (1991), ethanol was found to be weakly correlated to fullness in commercial beers. Other studies found that as ethanol concentration increased, both perceived viscosity and density in wine increased (Demiglio and Pickering, 2008, Gawel et al., 2007, King et al., 2013, Nurgel and Pickering, 2005). These results however, were not statistically significant, showing that there were other factors which were responsible for influencing perceived viscosity and density. Research has also shown that NABs have reduced palate fullness and mouthfeel (Langstaff et al., 1991, Malfliet et al., 2009). This is proposed to be not only due to the lack of ethanol, but also the production method used (discussed in section 1.3) suggesting that other factors may also contribute. There are still gaps in the literature looking at the effect of different ethanol concentrations on the body/fullness within a beer matrix.

1.5.3.3 Astringency

Astringency is described as the tactile sensation perceived by touch via mechanoreceptors (Breslin et al., 1993). The perception of astringency occurs in the mouth due to an interaction between polyphenols and salivary proteins, with tannins binding to salivary proteins and glycoproteins to form a layer that acts as a water barrier (Kielhorn and Thorngate Iii, 1999). This produces a sensation normally described as mouth-drying, puckering or rough mouthfeel.

Most studies have investigated the influence of ethanol on astringency in wines, as active astringent ingredients such as polyphenols are prevalent.

Research however, has not come to a full conclusion on the effect of ethanol on astringency perception. Some studies found that as ethanol concentration increased (from 0-15% v/v), the perception of astringency in wine decreased (Fontoin et al., 2008, Vidal et al., 2004). Conversely, increasing ethanol concentration was found in other studies to increase the perception of astringency, with increased palate dryness and a rough palate sensation (Demiglio and Pickering, 2008, Jones et al., 2008, King et al., 2013, Obreque-Slíer et al., 2010, Symoneaux et al., 2015). The decrease in astringency perception was proposed to be due to increased viscosity due to the physicochemical properties of ethanol (Pickering et al., 1998), as well as ethanol's limiting ability to form protein-tannin aggregates in wine. This contributed to lower precipitation, increased lubrication and reduced perceived astringency intensity (Green, 1993). Although some of these studies looked at ranges of ethanol concentrations found in beer, only one study has looked at the effect in a model beer system, showing that 4.5% ethanol was not a significant factor in the change in perception of astringency (Clark et al., 2011a). This could therefore show that the polyphenols in wine are more of an important factor in the interaction effect of ethanol and astringency perception, with these compounds found at lower concentrations in beer, or the concentration of ethanol in beer is too low to cause a significant interaction effect. More research is required to understand whether this is the case within a beer matrix.

1.5.3.4 Warming

Ethanol was found in early studies to cause irritation on the tongue, and this was described as a burning or stinging sensation (Clapperton, 1974, Green,

1987). To understand the mechanisms of this burning phenomenon, research looked into the burning sensation which occurs when alcoholic solutions were applied to skin wounds. It was found that even at low concentrations of ethanol (0.1 – 3% v/v), the transient receptor potential vanilloid receptor-1 (TRPV1) is activated, which is a multimodal nociceptor activated by both thermal and chemical stimuli, which also elicits the burning sensation found from capsaicin (Trevisani et al., 2002). In further studies, burning was the predominant attribute found on the circumvallate papilla of the tongue at 50% v/v ethanol (Allen et al., 2014). Nolden and Hayes (2015) used a general Labelled Magnitude Scale (gLMS) to assess perceived intensity of a range of ethanol concentrations found in beers, wines and spirits (4-48% v/v ethanol). They found similar results, with burning/tingling as the predominant sensation at 32 and 48% ethanol, yet at lower ethanol concentrations (between 4 and 16%) the dominant sensation was bitter (Nolden and Hayes, 2015). This research did not measure any ethanol concentrations below 4%, however it could be speculated that this warming effect may not occur within a beer matrix at lower ethanol concentrations. It should be noted, however that this study only measured the dominant sensations and therefore underlying sensations were not discussed.

Other research has looked further into these sensations and identified ethanol as an irritant in wine (8-13.5% v/v), with increased heat sensations dominant at higher ethanol concentrations. However, when wine was dealcoholized below 10% v/v, astringent became the dominant sensation (Meillon et al., 2009, Meillon et al., 2010). The only study to look at the warming sensation at lower ethanol concentrations includes research by Clark et al. (2011a) in a model beer system, who found that even at lower

concentrations of ethanol (0-4.5% ABV) warming perception is related to increased ethanol concentration. No studies to the authors' knowledge have looked at warming sensation in a real beer matrix.

1.5.4 Flavour

Delivery of volatile compounds retronasally occurs during consumption of a food or beverage. Volatile compounds pass from the pharynx, over the soft palate and into the nasal cavity (Linforth et al., 2002).

Many studies have looked at the influence of ethanol concentration on the flavour perception of wines (Baker et al., 2016, Jones et al., 2008, Meillon et al., 2009, Meillon et al., 2010) with one finding no significant difference in the attribute of 'overall flavour' (Jones et al., 2008). Others showed that as ethanol is removed from the wine through RO, 'red berries' flavour perception decreased (Meillon et al., 2009, Meillon et al., 2010). This however, could be due to the removal of more fruity flavour volatiles linked to this attribute via RO. Baker et al. (2016) found similar results, with increased ethanol concentration (10 and 15.5% v/v) characterised by 'spices' and 'dark fruit' flavours in wine.

Previous work has also looked at the impact of ethanol concentration on flavour attributes in beer. Clark et al. (2011a) found that ethanol concentrations ranging between 0 and 4.5% ABV in a model beer system did not influence sensorial perception of the flavour attributes 'sweaty/cheesy' and 'floral'. A significant difference was found however, with the attribute 'complexity of flavour', which increased with ethanol concentration. Missbach et al. (2017) also found differences in the flavour attributes of 'malty' and 'worty' in beers

with ethanol concentrations ranging from <0.5% to 5.4% ABV, with both of these attributes more pronounced in the lower alcohol beers. As is evidenced here, more research needs to be conducted on a range of flavour attributes in beer, to fully understand the role of ethanol in flavour perception.

1.5.4.1 Multimodal Flavour Perception

Multimodal refers to the fact that several senses are involved in flavour perception. Cross modal indicates that one modality can interact with another to modify the perception (Taylor and Roberts, 2004). Integration across sensory modalities has been found, with multimodal neurons receiving converging sensory information (Small and Prescott, 2005). Gustatory and olfactory interactions have been a focus of interest, with the development of functional neuroimaging techniques such as positron emission tomography (PET), functional magnetic resonance imaging (fMRI) and evoked related potential (ERP) mapping (de Araujo et al., 2003, Small and Prescott, 2005).

Multimodal flavour perception examples include aroma/taste interactions as well as taste/mouthfeel interactions. These have been shown to occur within alcoholic beverages. For instance, the presence of carbon dioxide has been shown to have implications on other sensory attributes, by significantly reducing sweetness perception (Clark et al., 2011a, Cowart, 1998, Hewson et al., 2009, Prescott et al., 2004, Symoneaux et al., 2015), enhancing sourness intensity (Comettomuniz et al., 1987, Cowart, 1998, Hewson et al., 2009, Prescott et al., 2004, Symoneaux et al., 2015), and producing a bitter aftertaste (Hewson et al., 2009). CO_2 also alters the perception of mouthfeel and aftertaste attributes, enhancing astringency (Hewson et al., 2009, Symoneaux et al., 2015) and bite, burn, numbing, warming and

carbonation/bubble pain (Green, 1992, Hewson et al., 2009, Kappes et al., 2006, McMahon et al., 2017, Clark et al., 2011a). This interaction was explained through competition between the trigeminal aspects of both factors, suppressing a warming sensation (Clark et al., 2011a) which has been found in previous studies with two trigeminal factors (capsaicin and ethanol) (Green, 1991). Interestingly no further work has been conducted considering this interaction effect, especially considering the potential to enhance the loss of the warming sensation found in NABs by increased carbonation. Interactions have also been found between glycerol (Gawel et al., 2007) and carboxymethylcellulose (Rolls et al., 2003). These were both found to have a depressive effect on alcohol palate heat, as this increases viscosity which shares a similar somatosensory pathway (Gawel et al., 2007, Jones et al., 2008, Rolls et al., 2003).

Ethanol is clearly a complex component of beverages, with interactions amongst other matrix components within wine and beer apparent. Therefore producing a NAB which creates the same sensory experience as its standard alcohol counterpart is a challenge.

1.6 Sensory Research

It is clear that robust sensory research is needed to understand the impact of ethanol on flavour perception in beer, as well as the sensory properties of NAB. As is highlighted throughout the introduction, there are numerous gaps in literature and these need to be explored to develop a comprehensive understanding of the role of ethanol within beer.

Different sensory techniques can be applied depending on the objective, which include discriminative, descriptive and affective analysis (Kemp et al., 2009). Discrimination tests determine sensory differences amongst samples and can be used by both trained panellists and naïve consumers (Kemp et al., 2009, Lawless, 2010, Rogers, 2017). Descriptive techniques on the other hand, identify the nature and magnitude of a sensory difference (Kemp et al., 2009). These methods require intensive and time consuming training with a group of assessors (6-18), who have been preselected due to their high levels of sensory acuity. The resulting data benefits from being highly analytical and therefore can be combined with instrumental data to understand the contributions of key components on sensory attributes, or combined with consumer data to understand drivers of liking (Kemp et al., 2018). The most common descriptive technique used is quantitative descriptive analysis (QDA) (Kemp et al., 2018, Stone et al., 2012a). In recent years, however, novel techniques such as rapid profiling have been adopted with untrained assessors, which limits time and cost and has been shown to produce reliable results when compared with more traditional approaches with a trained panel (Varela and Ares, 2014). These methods include Flash Profiling, Napping and sorted Napping, as well as Check-All-That-Apply (CATA) methodology (Ares and Jaeger, 2015). CATA is based on a multiple choice questionnaire in which respondents are asked to select all the options from a pre-defined list which they consider appropriate for a sample and has produced results similar to traditional descriptive analysis with a trained panel (Ares et al., 2010, Bruzzone et al., 2012, Dooley et al., 2010) and consumers (Jaeger et al., 2013a). Temporal methods capture dynamic changes during the consumption of the product. This is particularly

useful for products such as beer, as sensory properties change dynamically during oral processing. One example of this methodology is time intensity (TI), which measures the intensity of one attribute over time (Kemp et al., 2017). This method has also been used to capture temporal liking with consumers (Thomas et al., 2015). Over the years newer methods have been developed with the capability to capture more than one attribute over time. The first example of this was temporal dominance of sensations (TDS) (Pineau et al., 2009). Assessors are able to record the dominant sensations from an attribute list during product consumption (Pineau et al., 2009). More recently, Temporal Check-All-That-Apply (TCATA) was developed, with assessors checking all sensations that apply during consumption time, not just dominant sensations (Castura et al., 2016a), which benefits complex products where non-dominant attributes contribute to the sensory profile. These methods all typically use trained assessors, however promising data has been gathered using untrained assessors (Alexi et al., 2018, Ares et al., 2015, Bruzzone et al., 2012, Giacalone et al., 2016, Mello et al., 2019). Finally, affective tests are used with consumers to understand their subjective responses to products and can include both quantitative and qualitative methodologies. Quantitative methodologies include questions on preference, acceptance, choice and perceived intensity and liking of key sensory characteristics. Qualitative methodologies include focus groups, interviews or observation research. Both are vital in understanding consumer preferences, attitudes, opinions, behaviours and perception of products (Kemp et al., 2009). These tests are particularly important for understanding whether consumers can perceive differences in key attributes, help generate consumer relevant sensory terms and assess whether a product will be successful in the

market. In addition, launched products can be monitored to understand if there are routes for product optimisation or improvement (Kemp et al., 2009, Stone et al., 2012b). When selecting the most appropriate sensory methodology for a study, the advantages and disadvantages of using trained panels or naïve consumers' needs to be assessed.

1.6.1 Sensory Analysis of NABLABs

Only a handful of published studies to the authors' knowledge have used sensory and consumer methods to assess sensorial differences amongst beers with different ethanol concentrations.

Missbach et al. (2017) used temporal dominance of sensations (TDS) to understand changes in dominant flavour attributes of beers with varying ethanol content (<0.5-5.4% ABV) using trained panellists (n=10). Beers with different ethanol concentrations displayed some similarities in terms of 'bitterness', but 'worty-off flavour' was more pronounced in alcohol free beers early on in consumption, with 'malty flavour' increasing after swallowing. 'Bitterness' and 'astringency' dominated in higher alcohol beers after swallowing (Missbach et al., 2017). This study however, used commercially produced beers with no reference to their NAB production method, and therefore the reported results may be due to the changes in the overall beer matrix and not the absence of ethanol. In addition, the dominance of only five sensory attributes were assessed and so it is possible that important sensory attributes, which may not have been dominant during consumption, were not recorded.

Schmelzle et al. (2013) were the first to use a semi-trained panel (n=21) with descriptive analysis techniques, to understand differences amongst beers produced by different production methods. Twelve NABs (<0.5% ABV) were selected, which were produced by either physical, biological or mixed methods. In addition, consumer acceptance data was collected (n=116), using a subset of samples (n=9) to understand overall acceptance, as well as intensity of the tastes sweet, bitter and sour using a just-about-right (JAR) scale. The authors concluded that physical methods produced beers with 'sour' and 'bitter' taste, 'boiled cabbage-like' aroma and a 'mouth coating' texture. Biological and mixed methods produced beers which were divided into two categories; one with 'malty' and 'honey-like' aromas and another with a 'hop' aroma (Schmelzle et al., 2013). In terms of consumer acceptance, most samples were rated in the range of 'dislike slightly' to 'like slightly', showing no major preference for the NAB samples. Attributes 'boiled cabbage' and 'boiled potato' seemed to drive consumer disliking, with 'sweet' being a preferred attribute. Cluster analysis was performed, revealing two clusters of consumers with different overall liking, with one of these clusters found to enjoy the samples with intense hop aroma and sweetness, whilst also having markedly increased liking scores overall. However, this study used semi-trained consumers for the DA assessments raising questions on the robustness of the resulting data.

In a recently published study by Lafontaine et al. (2020), the sensory properties of 42 NABs (including lagers, pale ales, IPAs, hop water, radlers, ambers and wheat beers) were assessed by trained panellists (n=11) using a combination of descriptive analysis (DA) and CATA. American consumer

preference (n=129) was also performed on a subset of 12 NABs, assessing overall liking as well as separate scores for aroma, taste and mouthfeel. The trained panel were also asked to assess preference of the full sample set after DA - an unprecedented approach which is not recommended by sensory professionals and will therefore not be reported here. Overall, consumer liking of the subset of samples was low, with the most liked products (hop water and radler) only rated as 'slightly like' on the 9pt hedonic scale. Most other samples were rated as 'neither like or dislike' showing that consumers were indifferent. Contour plots were used to assess drivers of like/dislike and the attributes of 'thin', 'bitter' and 'malty', 'skunk' and 'stale' aroma were shown to drive consumer disliking. They were also found to be less satisfied with samples with 'hoppy', 'herbal', 'grassy', 'cheesy', 'black tea' aromas and 'bitter', 'astringent' and 'thin' mouthfeel. Liking scores were found to increase for NABs which had botanical aroma profiles ('hoppy', 'citrusy', 'tropical', 'stone fruit', 'melon', 'floral'). Although this study discussed the potential that certain beers could have been produced by different NAB production methods, due to their volatile and non-volatile matrix, the authors stated that these methods were unknown. Therefore it is hard to conclude which production methodologies produced beers with certain flavour characteristics. In addition, unfortunately this study did not cluster consumers to understand different groups of consumer preference. It was discussed that American consumers had the highest preference for 'sweet' NABs with 'citrusy', 'tropical' and 'stone fruit' aromas, however the overall liking was found to be low, showing that there is still a way to go in producing a NAB with high consumer preference.

Overall, this review of sensory analysis of NABs shows that research is limited in either its' quantity or quality. Further research is required utilising robust sensory methodology to understand the contribution of ethanol to the sensory properties of beer, whilst also investigating the impact of ethanol on physicochemical interactions. This will provide a sound basis for further studies investigating the impact of NAB production methods, developing a further understanding on improvements to the sensory quality of NAB.

1.7 Overview of Thesis Content

The objectives of this research were to: i) understand the sensory role of ethanol in beer; ii) determine physicochemical interactions between different beer styles, their influence on volatile release and influence of saliva; iii) investigate the physicochemical and sensorial properties of a range of commercially produced non-alcoholic lager style beers; iv) understand the effect of dealcoholizing beer by membrane technology on the sensory and physicochemical properties of different beer styles. Each chapter is written as a manuscript, which has either been published or are in press at the time of thesis submission.

The first experiment detailed in chapter 2 evaluates the influence of ethanol on consumer liking and perception of sensory characteristics in beer, as well as determining critical attributes driving consumer acceptance. This paper was published in Food Quality and Preference in September 2018. Chapter 3 explored the effect of ethanol concentration on volatile release in different beer styles to understand the mechanisms behind this complex process. This paper was published in Scientific Reports in November 2020. Chapter 4 investigated the physicochemical and sensorial properties of a range of commercially

produced non-alcoholic lager styles beers, whilst exploring possible effects of production method and other brewing parameters. Consumers also assessed their overall liking of these samples. This paper was published in Food Chemistry X in January 2021. Finally, Chapter 5 used reverse osmosis, a membrane based production process, to explore the effect of dealcoholizing two different beer styles on the physicochemical and sensorial attributes of the finished product. This paper was submitted to the Food Chemistry in December 2020. Chapter 6 draws conclusions based on findings from all experiments and gives recommendations for future research in the world of NAB.

2 Using a combined temporal approach to evaluate the influence of ethanol concentration on liking and sensory attributes of Lager beer

Preliminary thoughts Chapter 2:

One of the first steps in improving NAB is to understand the effect of ethanol on sensory properties such as taste, flavour and mouthfeel. Previously ethanol has been found to significantly affect sweetness, bitterness, sourness, carbonation, fullness/body, alcohol warming sensation and fruity flavour within ethanol/water solutions, wine matrixes and model beer systems (see section 1.5). Unfortunately no studies to date have investigated this effect within a real beer matrix, which is interesting considering predicted market values of NAB within the UK and Europe are expected to rise significantly in the next few years. This is also important as interactions with the product matrix due to multimodal flavour perception are likely to impact results.

Given that one of the barriers to NAB consumption reported by consumers is inferior sensory quality (taste/flavour/mouthfeel), research investigating drivers of liking and disliking for beers varying in ethanol concentration is needed to understand which properties drive these trends. The

following study was designed to investigate whether ethanol indeed affects consumers liking of beer, but also to explore the attributes which are affected when only ethanol is altered.

This chapter was published as a paper in Food Quality and Preference in September 2018:

Ramsey, I., Ross, C., Ford, R., Fisk, I., Yang, Q., Gomez-Lopez, J and Hort, J (2018) Using a combined temporal approach to evaluate the influence of ethanol concentration on liking and sensory attributes of lager beer. *Food Quality and Preference*, 68, 292-303.

doi: https://doi.org/10.1016/j.foodqual.2018.03.019

Using a combined temporal approach to evaluate the influence of ethanol concentration on liking and sensory attributes of Lager beer

Imogen Ramsey[ab], Carolyn Ross[c], Rebecca Ford[a], Ian Fisk[b], Qian Yang[a], Javier Gomez-Lopez[d], Joanne Hort[ae]

[a]Sensory Science Centre, Division of Food Science, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, United Kingdom

[b]Samworth Flavour Laboratory, Division of Food Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, United Kingdom

[c]Washington State University, School of Food Science, Pullman, WA 99164-6376, USA

[d] Campden BRI, Coopers Hill Road, Nutfield, Surrey, RH1 4HY, United Kingdom

[e]Riddet Institute, Massey University, Palmerston North, New Zealand

Key words: Beer, Ethanol, TCATA, Temporal Liking

Highlights:

- Ethanol concentration influences consumer temporal sensory characterisation of beer.
- Three patterns of consumer liking identified related to ethanol content in beer.
- Time point predicting overall liking varies by consumer segment and ethanol content.
- Temporal liking highlighted a reduction in liking for some products during aftertaste.
- Attributes driving temporal liking varied across consumer segments.

Abstract

A low alcohol beer evoking similar sensory enjoyment as its higher alcohol counterpart is an attractive proposition to breweries and consumers for increased sales volumes and health and societal reasons. This study aimed to determine the influence of ethanol on the temporal sensory characteristics and liking of beer as perceived by beer consumers. A commercial 0% ethanol concentration lager was spiked with ethanol to different concentrations (0.5%, 2.8%, 5% ethanol). Consumers (n=101) indicated their liking using temporal liking (TL) methodology (rated throughout consumption) and overall liking (rated at the end of consumption). Consumers also denoted the sensory properties perceived using temporal Check-All-That-Apply (TCATA). Overall, consumers were divided into 3 clusters with different patterns of liking. As ethanol concentration increased from 0 to 5%, the TL time that best predicted overall liking shifted from 60 sec to 10-20 sec indicating that liking of higher alcohol products was decided earlier on in consumption. Data suggested that in a lower ethanol beer, a liking judgement may not be stabilized until later in the evaluation, while in high ethanol beers, a liking judgement, either positive or negative, stabilised more rapidly. TCATA results revealed different temporal sensory profiles among the different ethanol concentrations. As ethanol concentration increased, the citation of sweetness, fullness/body and alcohol warming sensation increased. However, the relationship between TCATA citations and TL varied among the three clusters highlighting that, in relation to ethanol concentration, different negative and positive sensory drivers of preference exist for different segments of consumers.

2.1 Introduction

Beer consumers are accustomed to a product that offers a well-defined and complex taste (Blanco et al., 2016). In addition to these sensory considerations, the increasing interest of consumers regarding health and societal issues has motivated breweries to expand their portfolio of beers with low or no alcohol content products (SeekingAlpha, 2016, Rehm et al., 2016). As beer consumers are accustomed to particular attributes, the development of a low alcohol beer that displays a similar sensory profile to its higher alcohol counterpart is an attractive proposition. This would allow consumers to still enjoy the sensory properties of a beer while making responsible drinking choices (Missbach et al., 2017).

The challenge remains that sensory attributes in alcohol-free and alcohol-reduced beers differ from those in regular beer. Beers vary in their alcohol content but the majority of beers consumed contain between 3-8% ethanol (Preedy, 2011). Ethanol is an effective olfactory and trigeminal stimulus, contributing to the warming/burning perception of beer (Clark et al., 2011a, Green, 1987). Ethanol also contributes to the perception of different tastes, predominantly sweetness, bitterness and sourness (Hellekant et al., 1997, Martin and Pangborn, 1970, Scinska et al., 2000). Consuming beer is a multimodal experience and the influence of ethanol on sensory perception and its interactions with the other components in beer has been documented (Clark et al., 2011a). For example, ethanol interacts with hop acids to suppress a warming sensation at 4.5%, but also interacts with low levels of CO_2 to yield an increased alcohol warming sensation (Clark et al., 2011a). Furthermore, ethanol has been found to physically influence aroma release in beer during

consumption (Clark et al., 2011b). The influence of ethanol concentration on dynamic headspace recovery of different volatile compounds in ethanol/water solutions using proton transfer reaction mass spectrometry (PTR-MS) with concentrations similar to those found in beer (0, 2.5 and 6.2% v/v) showed that increased ethanol concentration decreased volatile release (Aprea et al., 2007). This reported similar findings to Clark et al. (2011b), again with dynamic headspace, with the change being attributed to an increase in the solubility of aroma compounds (Aprea et al., 2007, Conner et al., 1998, Perpete and Collin, 2000). Ethanol clearly has the capability to impact sensory perception of beer. Therefore, an understanding of how ethanol reduction in beer affects consumer perception and acceptance is important (Kaneda et al., 2002, Porretta and Donadini, 2008). Previous studies have reported that consumers can distinguish among beers containing different ethanol concentrations. For example, in one triangle test, consumers could distinguish between an alcohol free (0.5% ethanol) and regular (5% ethanol) beer but interestingly were not able to identify which was of a higher alcoholic strength, suggesting consumers are not necessarily aware of the characteristics associated with ethanol (Lachenmeier, 2014). In another study, consumers were able to distinguish between an alcohol-reduced (3.8% ethanol) and regular beer (5.3% ethanol), with the standard strength beer having more overall appeal than the lower strength (Segal and Stockwell, 2009). However, these studies did not report consumer liking of the products, which is an important piece of information for innovating a commercially successful product.

Beer possesses a highly complex sensory profile (Clark et al., 2011a) and as with other beverages including wine (Baker et al., 2016), displays a

temporal aspect. In short, beer perception changes over the consumption period, from the moment the beer is placed in the mouth to when the final sensations of that beer, including aftertaste, abate. Particularly, the sensory attributes of beer arising from the presence of ethanol (alcohol warming sensation) and iso-alpha acids (bitter taste) are well documented to have a temporal quality in beer (Arrieta et al., 2010). Thus to better understand consumer perception of a low-alcohol beer, the application of temporal methods is important. Previous testing of the temporal sensory aspects of beer has relied upon the use of time intensity or dominance testing using Temporal Dominance of Sensation (TDS) (Missbach et al., 2017), and usually with trained panels. Using TDS, differences among three beers based on their ethanol concentration with trained panellists were identified. Beer samples containing <0.5%, 3.4% and 5% ethanol displayed differences in the dominance of astringency and other fermentation-related flavours, with the higher ethanol concentrations showing increased bitterness and astringency (Missbach et al., 2017). However, it is unclear what impact this might have had on consumer liking.

Understanding the sensory attributes that drive consumer liking of food and beverage products is critical to both the food and beverage industry. In the present study, the impact of ethanol concentration on the perception of beer was investigated with consumers using a combination of methods to evaluate temporal and overall liking and the temporal perception of key sensory attributes. Temporal Check-All-That-Apply (TCATA) methodology (Baker et al., 2016, Castura et al., 2016a) was chosen over TDS as it does not limit evaluation to just dominant attributes. Previous studies have successfully

employed similar methods to determine drivers of liking (Ares et al., 2017, Thomas et al., 2015); however, no studies have yet examined temporal liking in beer.

The objectives of this study were therefore to i) evaluate the influence of ethanol on consumer liking of lager and perception of its sensory characteristics; ii) determine if particular time points during temporal liking related to overall liking; and iii) investigate the relationship between the temporal sensory profile of beer and temporal liking data identifying critical attributes driving consumer acceptance of beer in relation to ethanol concentration.

2.2 Methods

2.2.1 Participants

Consumers (n=101: 53 men, 48 women; aged 19-70 (mean age 32)), who self-reported consumption of beer at least once every two months, participated in this study. Data concerning frequency of consumption and the types of beer consumed was also obtained. Approval from the University of Nottingham Medical Ethics Committee was granted before the study commenced and the subjects were offered an inconvenience allowance to participate.

2.2.2 Beer Samples

A 0% ABV lager style beer (Carlsberg, Northampton, UK) was used as a base beer from which four experimental beer samples (0, 0.5, 2.8 and 5% ethanol) were prepared. These ethanol concentrations were selected to reflect a

full ethanol beer (5%), an intermediate ethanol concentration (2.8%), a low ethanol beer (0.5%), and an alcohol free beer (0%). In the United States, an alcohol-free beer is described as having 0% ethanol concentration, a non-alcoholic beer corresponds to a beer containing 0.5% ethanol or less and a lower alcohol beer contains less than 3.5% ethanol. In the United Kingdom, alcohol duty rates are increased when a beer exceeds 2.8% ethanol concentration and so some brewers try to satisfy this target for their lower alcohol beers (Branyik et al., 2012). The above points were considered when selecting the specific concentrations to represent ethanol concentrations of beer commercially available in each of these categories.

To create the 0.5, 2.8 and 5% ethanol samples, 1.7, 9.6 and 17.5 mL of 99.5% food grade ethanol (VWR International, Lutterworth, UK) and 28.3, 20.4 and 12.5 mL of still water (Danone, Paris, France) were added, respectively, to 300 mL of beer. The 0% ethanol beer also had 30 mL of water added to ensure that all samples were treated the same. Commercial bottles of beer (330 mL) stored at 4 ±1°C, were opened as close to sample testing as possible, 30 mL was poured out of the bottle, and the relevant ethanol/water solution was added back in after which the bottle was inverted to ensure adequate mixing. Beer samples (30 mL) were poured into plastic serving cups and were used within 20 mins of opening. This approach was used to minimise sample handling and limit the decarbonation and volatilisation of the samples.

2.2.3 Sensory Attributes

Attributes and definitions for beer evaluation were developed in reference to published literature (Langstaff and Lewis, 1993, Martin and

Pangborn, 1970, McMahon et al., 2017, Meilgaard et al., 1979) as well as through the use of a naïve panel of six beer consumers.

2.2.4 Procedure

All consumers participated in two evaluation sessions over two weeks at the Sensory Science Centre, Sutton Bonington campus, University of Nottingham. Both sessions began with a familiarisation session (15 min) after which consumers evaluated samples in isolated sensory booths (45 min). Consumers evaluated temporal liking (TL) first and overall liking (OL) second to gain an understanding of consumer liking of the product during specific periods of consumption (before swallow and aftertaste) and then an overall score. TL and OL were evaluated in session one and sensory attributes using TCATA in session two. Although not always shown to cause bias (Jaeger et al., 2013b) this order was chosen to avoid analysis of sensory attributes influencing liking results as reported in other studies (Earthy et al., 1997, Popper et al., 2004).

2.2.4.1 Familiarisation Sessions

Previous research has shown that a short familiarisation session (7-10 mins) can result in a small increase in consumer ability to discriminate among samples (Jaeger et al., 2017). In session one familiarisation involved the explanation and practice of the evaluation protocol for TL and OL. In session two, the TCATA method was described to the consumers as a relatively new technique, and the importance of checking and unchecking perceived attributes during evaluation was discussed (Castura et al., 2016b). The attributes (Table 2.1) were also reviewed to ensure consumers understood them all.

Table 2 .1: *TCATA attributes and definitions provided to consumers during familiarisation session.*

Flavour and Taste Attributes	**Definition**
Malty Flavour	Smell and taste of malty cereals. Can be related to smell of Ovaltine drink.
Hoppy Flavour	Smell and taste of hops which can be flowery and herbal.
Fruity Flavour	The aroma and taste of fruit characteristics – including banana, apple, pineapple, peach, lemon, orange.
Bitter Taste	Taste stimulated by strong black coffee, beer, red wine or tonic water.
Sweet Taste	Taste stimulated by sugar when experienced in mouth.
Sour Taste	Taste stimulated by acids when experienced in mouth.
Fullness/Body	Feeling of thickness/fullness as beer is moved around in the mouth.
Alcohol Warming Sensation	The feeling of warming which is characteristic of ethanol throughout the mouth.
Tingly Sensation	Perception of irritation such as prickling, stinging and bubbles bursting in mouth from carbonation. The feeling of pins and needles.
Astringent Mouthfeel	The feeling in mouth of roughing, puckering and drying.

For all in-mouth evaluations, the in-mouth protocol remained the same: consumers were asked to place the sample in the mouth and press the green start button immediately, move the sample around in the mouth and then swallow at 10s when a prompt appeared on-screen. Although not necessarily normal drinking behaviour, this enabled the protocol to be controlled and facilitated comparison between TL and TCATA data. Consumers continued the

evaluation up to 60s, at which point it ceased. If nothing was perceived before reaching the end of the evaluation time consumers were told to deselect attributes. Consumers were given a handheld tablet (Apple, Cupertino, California, USA) and practice sample at the end of each familiarisation session so that they could interact with the method and software prior to formal evaluations.

In each session all samples (n=4) were presented monadically under Northern hemisphere lighting using a randomised balanced design according to a Williams Latin Square (Meyners et al., 2013). Data were captured using Compusense© Cloud software (Guelph, Ontario, Canada). To minimise fatigue and carryover, consumers were given a forced 2 min break between each sample, and were told to take at least 2 sips of water (Evian, Danone, France) during this break to cleanse the palate.

2.2.4.2 Temporal Liking Measurement

During the first session, consumers used a 15-cm semi-structured line scale, anchored with dislike extremely and like extremely to continuously quantify their current liking. During the 60s evaluation time, consumers were instructed to click on the scale at any point that their perceived liking changed. The total duration of evaluation (60s) was established through preliminary investigations as a duration that was adequate to capture relevant changes in aftertaste perception while minimising fatigue to the consumers. Data was recorded at one data point per second.

2.2.4.3 Overall Liking Measurement

Within 30s of completing the TL measurement, consumers assessed their overall liking of the sample using a 9-pt hedonic scale ranging from 'dislike extremely' to 'like extremely'.

2.2.4.4 Temporal evaluation of sensory attributes in mouth using Temporal Check-All-That-Apply (TCATA)

In the second session, consumers assessed the presence of 10 attributes within each sample. Prior to the test, consumers were instructed to familiarise themselves with the position of the attributes on screen, which were presented in a three-column format. The attribute order was randomised across subjects to balance bias associated with list order but was retained for a given panellist (Meyners and Castura, 2016).

2.2.5 Instrumental Analyses

Instrumental analyses were conducted to record the impact of ethanol concentration on key chemical characteristics. The ethanol content, density and specific gravity were all measured in triplicate across sample bottles prepared as described in section 2.2.2, using an Anton Paar Alcolyzer and DMA4500 (Graz, Austria). The pH of all samples was determined using a Metler Toledo FiveGo pH meter (Columbus, Ohio, USA) and the titratable acidity (TA) measurements were made using a Metrohm 702 SM Titrino potentiometric titrator (Metrohm UK Ltd, Cheshire, UK) after calibration with pH 4.0 and 7.0 standards. To determine if differences existed between samples, an ANOVA was performed followed by a comparison of means calculated by Tukey's Honest Significant Difference (HSD) post-hoc test (XLStat 19.01, Addinsoft, New York, USA).

2.2.6 Data Analyses

An α risk of 0.05 was set as the level of significance in all data analyses.

2.2.6.1 Overall Liking

To determine if differences existed between samples in terms of overall liking a mixed model two-factor ANOVA (sample, panellist), with panellist as a random effect was performed followed by a comparison of means calculated by Tukey's HSD post-hoc test (XLStat 19.01, Addinsoft, New York, USA). To ascertain if liking patterns varied across consumers a cluster analysis (XLStat 19.01, Addinsoft, New York, USA) on overall liking data was performed using agglomerative hierarchical clustering employing a dissimilarity matrix with Euclidean distance and Ward's method in the agglomeration (Desai et al., 2013). Further analysis was then performed, with a two-factor ANOVA (as above) to examine differences between samples within each cluster. Cluster membership was further explored according to the demographic variables collected in this study using a Chi square analysis and Fishers exact test (Gellynck et al., 2009).

2.2.6.2 Temporal Liking

For each product and consumer, six liking scores were extracted from the temporal data i.e. every 10s until 60s. As the cluster analysis discovered 3 different patterns of liking the temporal liking data was assessed taking different clusters into account. For each cluster, a two-factor ANOVA (sample and time point) with liking as the dependent variable was then performed (XLStat 19.01, Addinsoft, New York, USA). Tukey's HSD tests were

subsequently used to identify where significant differences occurred between time points and clusters.

2.2.6.3 Relating Temporal liking to Overall Liking

Liking data were extracted for all time points, however only data relating to 10, 20, 40 and 60s were subsequently further analysed as no differences in liking were found at 30 and 50s. These liking data were modelled against overall liking which had been determined after the 60s evaluation period had ceased (Table 2.5). In order to determine if particular time points during TL related to overall liking, an ordered probit model was employed (Stata 14.0 (Stata Corp, College Station, TX, USA)). This model was selected because the dependent variable was an ordered scale, ranging from 1 to 9 (Long, 1997). A separate model was estimated for each consumer cluster at temporal liking times of 10 (swallow), 20, 40 and 60s (end of test) to identify which time point best related to the overall liking.

2.2.6.4 Analysis of TCATA data

2.2.6.4.1 Analysis of Average Proportions of Citations

The analysis of the average proportion of citations followed a similar method as McMahon et al. (2017), with each attribute being assessed as the proportion of the 60s time period in which it was selected (XLStat 19.01, Addinsoft, New York, USA). For example, if malty was checked for a duration of 15s and hoppy for 25s, the average proportion of citations would be 15/60 = 0.25 for malty and 25/60 = 0.42 for hoppy.

2.2.6.4.2 TCATA Curves

Following a similar procedure as described in Castura et al. (2016a); and McMahon et al. (2017), data were exported for each attribute at 0.1s intervals in the form of either '1' or '0' to show presence or absence of this attribute. Proportions of citations were calculated as the percentage of panellists who perceived (or checked) an attribute at any given moment during the evaluation period. For each attribute, TCATA curves (smoothed using the cubic spline function in R (The R Foundation, Vienna, Austria) to reduce noise in the data (McMahon et al., 2017)) were calculated per treatment at each time point (each 0.1 s during the evaluation period). Thicker sections of an attribute line were used to represent segments where the proportion of citations was significantly different in contrast to the other samples. The average proportion of citation of the attribute for the other samples was plotted on the same figure, when significant, using a dotted line enabling visualisation of the direction of the difference i.e. higher or lower citation, and the time periods during which significant differences were observed.

2.2.6.4.3 Multivariate Analysis of TCATA Attributes

The relationship between beer samples and TCATA attributes was investigated using principal component analysis (PCA) on unfolded data, to create a two-way matrix with sensory attributes in columns and rows corresponding to sample (ethanol concentration) by time point (Castura et al., 2016a, Castura et al., 2016b) (Stata 14.0 (Stata Corp, College Station, TX, USA)). PCA plots were constructed to show how attributes were perceived and evolved in relation to treatments (McMahon et al., 2017).

2.2.6.5 Relationships between temporal sensory attributes (TCATA) and temporal liking (TL)

To evaluate the contribution of each TCATA attribute to temporal liking, a random effects regression model was used (Stata 14.0 (Stata Corp, College Station, TX, USA)). This analysis was selected so as to compare, by panellist, the evaluation of the same attribute at different points in time. Because the same panellist is evaluating the same attribute at various points in time, the evaluations of that panellist are correlated with each other. A random effects model takes into account this non-independence among the observations. For this model, TL was the dependent variable whilst the TCATA attribute (i.e. astringent, malty, etc.) was used as the independent variable, with z-values showing whether this was a positive or negative association.

2.3 Results

2.3.1 Instrumental Analyses

The instrumental analyses confirmed that the planned concentrations of ethanol were achieved. The ANOVA showed that the effect of ethanol concentration was significant ($F(3, 11) = 897$, $p=<0.0001$) as were associated specific gravity ($F(3, 11) =67.8$, $p=<0.0001$) and density values ($F(3, 11) = 69.1$, $p=<0.0001$) (Table 2.2). Analysis of the pH values of the samples, although close, were significantly affected ($F(3, 87) =2.83$ $p=0.043$) with the Tukey test indicating the 0% and 0.5% having a significantly higher pH compared to the 5% ethanol sample (<0.05). The analysis of variance showed that TA was significant across samples ($F(3,11) = 35.8$, $p=<0.0001$), whereby

the Tukey test indicated 0% and 5% were significantly different ($p<0.05$), although still quite close in absolute value (differential= 0.703g/L). Theoretically, this increase in acidity might have increased the citation of the sour attribute in the TCATA for the 5% sample, however this was not found.

Table 2. 2: *Mean (3 replicates) chemical profile of the beer samples.* ^{abcd}*different letters within a column represent a significant difference at p<0.05 (Tukey's HSD)*

Beer Sample	**Alcohol by volume (ABV%)**	**pH**	**Density (g/cm³)**	**Specific Gravity (SG)**	**Titratable Acidity (g/L)**
0% Ethanol	0.06 ^{d}	4.209 ^{a}	1.019 ^{a}	1.021 ^{a}	0.848 ^{c}
0.5% Ethanol	0.64 ^{c}	4.202 ^{a}	1.018 ^{b}	1.020 ^{b}	1.130 ^{b}
2.8% Ethanol	2.85 ^{b}	4.185 $^{a\,b}$	1.015 ^{c}	1.017 ^{c}	1.260 ^{b}
5% Ethanol	5.25 ^{a}	4.175 ^{b}	1.012 ^{d}	1.014 ^{d}	1.551 ^{a}

2.3.2 Overall Liking

ANOVA revealed no significant differences (F (3, 403) = 0.426, p =0.735) among the four beer samples in terms of overall liking. However, agglomerative hierarchical clustering analysis was subsequently performed and three clusters of consumers were identified.

Table 2.3 shows the average overall liking scores of the three consumer clusters. The ANOVA yielded significant differences for the interaction between sample identity and cluster (F (2, 6) = 15.2, p=<0.0001), indicating

that the overall liking of the samples varied with the consumer cluster. Statistically, scores for cluster 1 (C1, n=23) showed significant differences for consumers liking (F (3,91) = 15.7, p=<0.0001) with Tukey test indicating that the overall liking was significantly higher for the 5% beer compared to the 0%, 0.5% and 2.8% samples, which were 'disliked slightly' ($p<0.05$). Cluster 2 (C2, n=50) showed no significant difference in overall liking among the samples (F (3, 199) = 0.913, p=0.436), but rated all samples higher than the other clusters as either 'like slightly' or 'like moderately'. The ANOVA for cluster 3 (C3, n=28) yielded significant differences for consumer liking (F (3,111) = 14.5, p=<0.0001) with the Tukey test revealing that the overall liking for the 0%, 0.5% and 2.8% was significantly higher than for the 5% beer, which was rated as 'dislike very much' ($p<0.05$). Interestingly consumers in this cluster disliked all beer samples.

Table 2. 3: *Overall mean liking scores for beer samples by cluster. Different letters within a cluster^{ab} or beer sample^{AB} represent a significant difference in liking (Tukey's HSD ($p<0.05$))*

Beer Sample	**Cluster 1 (n=23)**	**Cluster 2 (n=50)**	**Cluster 3 (n=28)**
0% Ethanol	4.04^{bB}	6.78^{aA}	4.04^{aB}
0.5% Ethanol	4.57^{bB}	6.44^{aA}	4.29^{aB}
2.8% Ethanol	4.00^{bB}	6.72^{aA}	4.96^{aB}
5% Ethanol	6.65^{aA}	6.32^{aA}	2.32^{bB}

Cluster membership was further explored according to the demographic variables collected in this study which included beer consumption patterns, gender, age and types of beer consumed (e.g. ale and non-alcoholic beer) but low cell numbers meant no inference could be made regarding their effect on cluster membership. In addition to this, the familiarity of beer styles (more specifically non-alcoholic beer) over all consumers was studied, but no significant differences were found to suggest that non-alcoholic beer drinkers rated the 0% sample higher, as might be expected.

2.3.3 Temporal Liking

Because of the different patterns of liking found among consumers in overall liking, subsequent analyses looked at each cluster separately. Figure 2.1 shows the average temporal liking curves for each sample by cluster. In general, they show that temporal liking of the beer samples in each cluster reflected those results seen in the overall liking (Table 2.3). The ANOVA showed that the effect of ethanol concentration on liking was significant (F (3, 91) = 15.7, p=<0.0001) for C1, and the Tukey test showed a significantly higher and constant level of liking for 5% ethanol sample over the entire 60s evaluation period ($p<0.05$). Some reduction in liking for the other three samples was evident around and after swallowing. No significant differences were found in liking scores between samples for C2 (F (3, 199) = 0.913, p=0.436) and, visually, the level of liking was generally consistent throughout the evaluation. C3 generally showed consistent dislike for most of the samples throughout the temporal evaluation, as seen with the overall liking data. Again ANOVA showed that there was a significant difference in terms of liking between samples (F (3, 111) = 14.5, p=<0.0001), with the tukey test indicating

the 0% sample scoring significantly higher for the duration. This cluster also clearly disliked the 5% sample the most, particularly after swallowing ($p<0.05$). The ANOVA performed to compare liking for each sample within a given cluster at each increasing 10s of the evaluation time highlighted some of these differences between the samples. For C1 and C2, no significant differences were found. However, for C3, a difference was found for the 5% ethanol beer (F (5, 143) = 4.31, p=0.001), with the Tukey test showing a significant decrease in liking when assessed at latter time points (40, 50 and 60s), during the aftertaste, compared to the first point which was in mouth, at 10s ($p<0.05$).

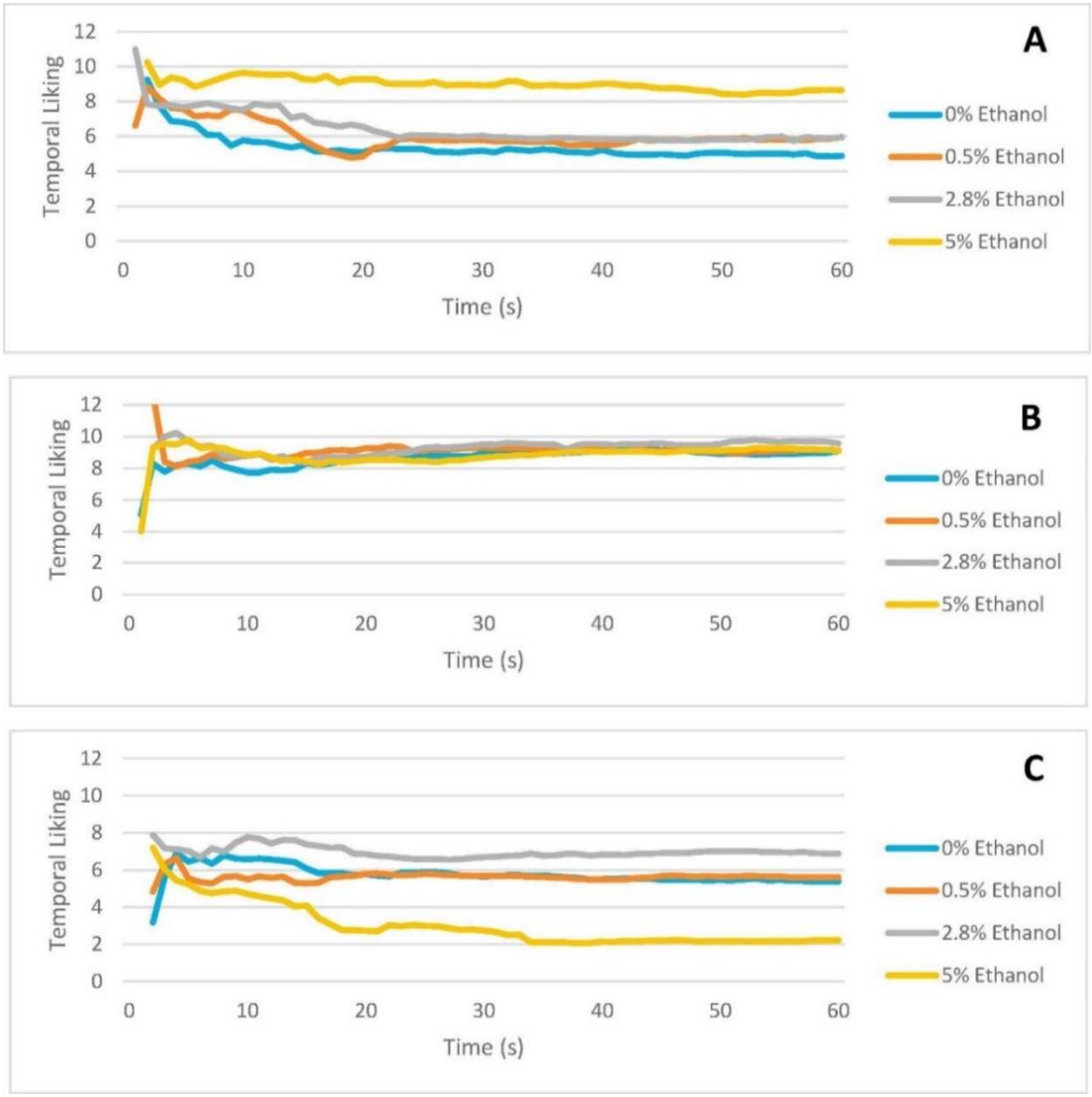


Figure 2. 1*: Temporal liking curves for Cluster 1 (A), Cluster 2 (B) and Cluster 3 (C) showing the mean liking of each beer sample by cluster.*

2.3.4 Relating Temporal Liking to Overall Liking

The relationship between liking at a given time point (determined using TL) and overall liking determined at the end of the test (using a 9-pt hedonic scale) was assessed and although clusters showed similar trends there were differences and hence the data was interrogated by cluster (Table 2.4).

The ordered probit estimates revealed that the time point from the TL data that best predicted overall liking varied with beer sample and cluster. For 0% ethanol, TL at 60s (the end of the evaluation) best predicted overall liking in both C1 ($p=0.015$) and C2 ($p=0.006$). None of the TL evaluations significantly predicted overall liking in C3. . For 0.5% ethanol, TL at 60s again best predicted overall liking in C1 ($p=0.049$). For C2, overall liking was significantly predicted by liking at both 40 ($p=0.001$) and 60s ($p=0.001$). Again, evaluations at none of the time points was a significant predictor of overall liking for C3. For 2.8% ethanol, overall liking for both C1 ($p=0.014$) and C2 ($p=0.009$) was significantly predicted by TL at 40s. No significant time point was found for C3. Finally, for 5% ethanol, overall liking for C1 was significantly predicted by evaluations at 10 ($p=0.005$) and 60s ($p=0.041$). For C2 ($p=0.005$) and C3 ($p=0.002$), overall liking was significantly related to liking at 20s.

To a certain extent, as ethanol content decreased, overall liking was better predicted by temporal liking increasingly later in the consumption process. For cluster 3, who did not really like any beers, it was more difficult to find a temporal point relating to OL except for the 5% beer. In this beer, evaluations early in the consumption process better predicted overall liking.

Table 2. 4: *Ordered probit coefficients and associated p values illustrating the relationship between overall liking (9-pt hedonic scale) and temporal liking (15-cm line scale) for all consumer clusters and beer samples at 10, 20, 40 and 60 seconds of evaluation. Bold font indicates significant relationships ($p<0.05$).*

0% Ethanol Beer						
	Cluster 1		Cluster 2		Cluster 3	
Evaluation time (s)	coefficient	p-value	coefficient	p-value	coefficient	p-value
10	0.161	0.191	-0.105	0.114	0.011	0.949
20	0.214	0.130	0.165	0.081	0.155	0.716
40	-0.183	0.426	0.156	0.076	0.648	0.468
60	0.528	**0.015**	0.260	**0.006**	0.553	0.331
0.5% Ethanol Beer						
	Cluster 1		Cluster 2		Cluster 3	
Evaluation time (s)	coefficient	p-value	coefficient	p-value	coefficient	p-value
10	-0.056	0.663	-0.054	0.519	0.05	0.842
20	0.243	0.1	0.029	0.801	-0.189	0.708
40	0.100	0.681	0.446	**0.001**	0.979	0.319
60	0.392	**0.049**	0.321	**0.001**	0.801	0.328
2.8% Ethanol Beer						
	Cluster 1		Cluster 2		Cluster 3	
Evaluation time (s)	coefficient	p-value	coefficient	p-value	coefficient	p-value
10	-3.3×10^{-6}	1	0.857	0.289	0.281	0.809

20	-0.13	0.4	0.109	0.272	0.471	0.151
40	0.80	**0.014**	0.336	**0.009**	-0.363	0.569
60	-0.589	0.841	0.119	0.282	0.636	0.192
5% Ethanol Beer						
	Cluster 1		Cluster 2		Cluster 3	
Evaluation time (s)	coefficient	p-value	coefficient	p-value	coefficient	p-value
10	0.528	**0.005**	-0.28	0.676	0.051	0.622
20	0.526	0.066	0.253	**0.005**	0.672	**0.002**
40	-0.763	0.114	0.117	0.379	-0.261	0.638
60	0.780	**0.041**	0.258	0.032	0.821	0.163

2.3.5 Impact of Ethanol Concentration on Temporal Perception of Sensory Attributes (TCATA)

2.3.5.1 Analysis of Average Citation Rates for Temporal Data

The average proportion of citations of various attributes varied among the beer samples as analysed using Cochran's Q analysis (Table 2.5). The citation of the mouthfeel attributes of fullness/body and alcohol warming were higher in the 5% ethanol sample compared to the 0, 0.5 and 2.8% ethanol samples ($p<0.05$). In the citation of the sweet attribute, the 5% ethanol sample was higher than the other three samples, with significant differences also observed between the 0 and 2.8% ethanol samples.

Table 2. 5: *Average proportion of consumer panel citations of TCATA sensory attributes.* [abc]*Different letters within a column represent significant differences among samples (Fisher's Exact Test (p<0.05)).*

	Flavour Attributes			Taste Attributes			Mouthfeel Attributes			
Beer Sample	Malty	Hoppy	Fruity	Bitter	Sweet	Sour	Fullness/Body	Alcohol Warming	Tingly	Astringent
0% Ethanol	0.39	0.18	0.18	0.32	0.23 [c]	0.17	0.08 [b]	0.06 [b]	0.22	0.20
0.5% Ethanol	0.35	0.22	0.17	0.31	0.29 [bc]	0.18	0.13 [b]	0.04 [b]	0.21	0.16
2.8% Ethanol	0.37	0.16	0.19	0.31	0.36 [b]	0.13	0.13 [b]	0.09 [b]	0.22	0.17
5% Ethanol	0.31	0.17	0.25	0.27	0.48 [a]	0.14	0.19 [a]	0.17 [a]	0.25	0.15

2.3.5.2 TCATA Curves

Differences were observed among the samples in the citation of sensory attributes over time (Figure 2.2). For the 0% ethanol sample, in general, fewer attributes were cited compared to the other three samples. Between ~14 and 60s, fullness/body was cited significantly less frequently ($p<0.05$) compared to the three other ethanol concentrations, as well as sweet taste and fruity flavour from ~4 to 60s. The warming attribute was cited significantly less often ($p<0.05$) compared to the three other ethanol concentrations at ~26s and ~30s, within the 0% ethanol sample, however, interestingly it was not at zero which may have been expected suggesting other attributes may contribute to its perception in beer.

For the 0.5% ethanol sample, several significant differences in the citations of attributes were found. Compared to the other 3 beer samples, sweetness was cited significantly less frequently ($p<0.05$) from ~4 to 60s and malty flavour from ~20 to 60s. Alcohol warming sensation was also cited significantly less often from ~21 to 60s and bitter taste from ~16 to 20s ($p<0.05$). For the 2.8% ethanol sample, bitter taste was cited significantly less frequently from ~15 to 23s and ~27 to 44s. From ~16 to 24s, malty flavour was perceived less often ($p<0.05$).

For the 5% ethanol sample, attributes were cited more frequently compared to the 0 and 0.5% ethanol samples. Malty flavour was cited less often ($p<0.05$) from ~15 to 60s and bitter from ~16 to 60s. Sour was highlighted as an attribute being cited significantly less ($p<0.05$) from ~30 to 40s and hoppy flavour from ~25 to 37s. Alcohol warming sensation was cited significantly more often ($p<0.05$) in the 5% beer between ~55 and 60s.

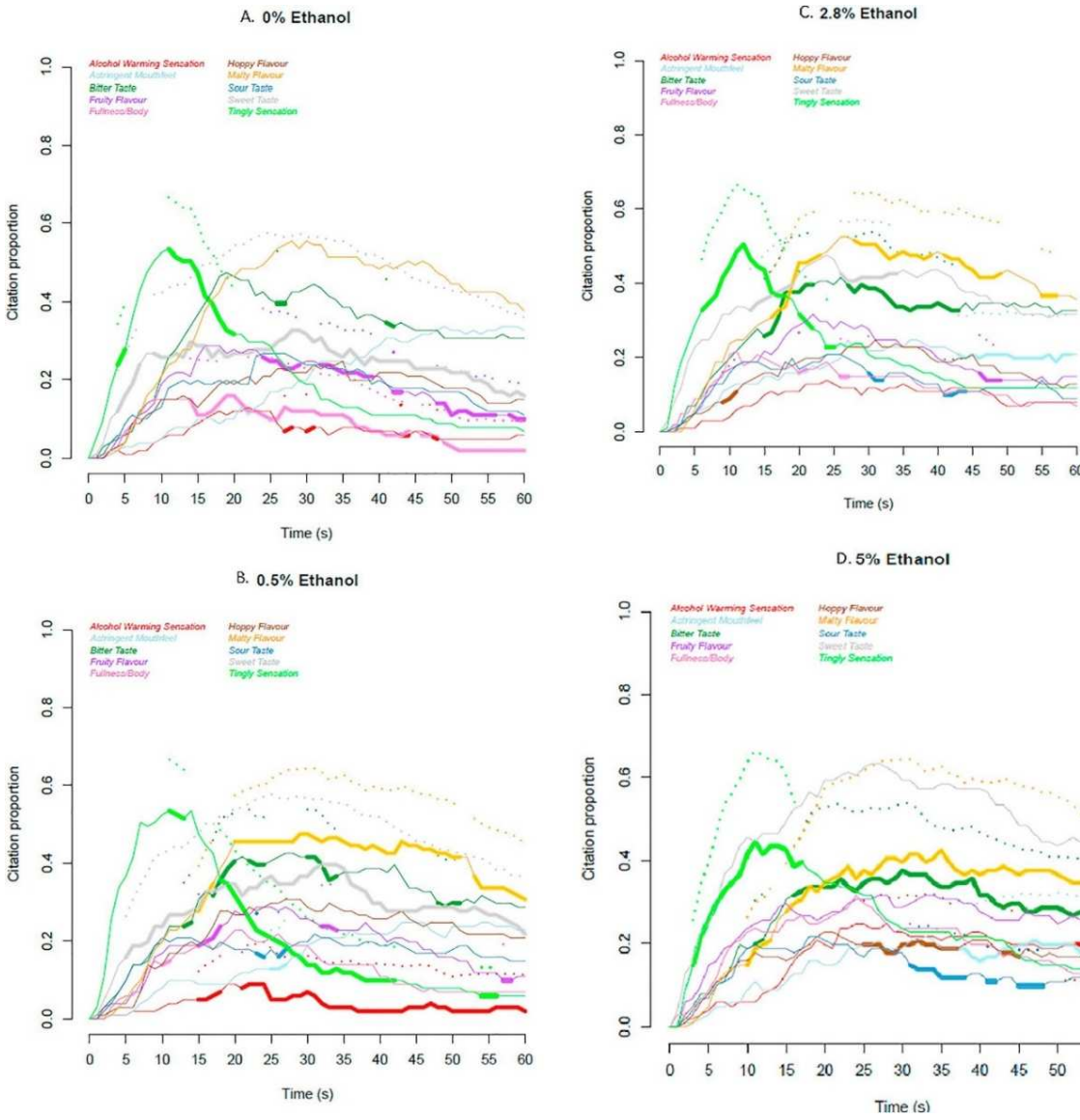


Figure 2. 2*: Smoothed TCATA attribute curves (continuous lines) for A: 0% ethanol; B: 0.5% Ethanol; C: 2.8% ethanol and D: 5% ethanol. Thicker segments represent time period where proportion of citation is significantly different to the other 3 samples. In contrast, dotted lines represent pooled average proportion of citations for the other 3 samples, where significantly different ($p < 0.05$). Each attribute is represented by a different colour.*

As ethanol concentration increased attributes were cited more frequently. The lower ethanol concentration samples were cited significantly less compared to the other samples for sweetness, fullness/body and alcohol warming sensation. For the higher ethanol concentration sample, alcohol warming sensation was cited significantly more often compared to all other samples.

2.3.5.3 Multivariate Analysis of TCATA Attributes

The ethanol content in the beer clearly influenced the temporal citation of flavour, taste and mouthfeel sensory attributes. The influence of ethanol content described above is clearly visualised through the use of a PCA (Figure 2.3), showing the multivariate space and the temporal evolution of attributes in the beer samples over the 60s evaluation period. Ethanol concentration is labelled at the 40s evaluation point. The two components accounted for 83.05% variation in the data. PC1 is strongly correlated to bitter (0.934), malty (0.918), hoppy (0.866) and fruity (0.858), whereas, PC2 is strongly correlated with tingly sensation (0.902) and fullness/body (0.758) and negatively correlated with astringent (-0.568). The trajectories for each beer sample start at the top left (t=0) where the citation rate for all attributes is 0. As this biplot is not a continuous loop, it shows that consumers were still perceiving attributes up until the end of the evaluation at 60s. As evaluated by citation frequency, the early onset attributes in the beer samples were tingly, fullness and sweet occurring around~10s. The delayed onset attributes, appearing at ~45s, were identified as astringent and malty and they were more associated with the beer aftertaste.

When comparing the beer samples in their temporal evolution, the 0 and 0.5% ethanol samples displayed similar profiles, as the trajectories show these samples initially described as tingly, evolving to become more sour and ending with being described as having malty and astringent aftertastes. The 2.8% ethanol sample again was initially described as tingly, however there was a more delayed onset of alcohol warming sensation and fruity, finishing with bitter and hoppy aftertastes. The 5% ethanol sample was initially described as tingly, but also displayed delayed onset attributes of fullness, sweet, fruity and warming, with a sour and hoppy aftertaste.

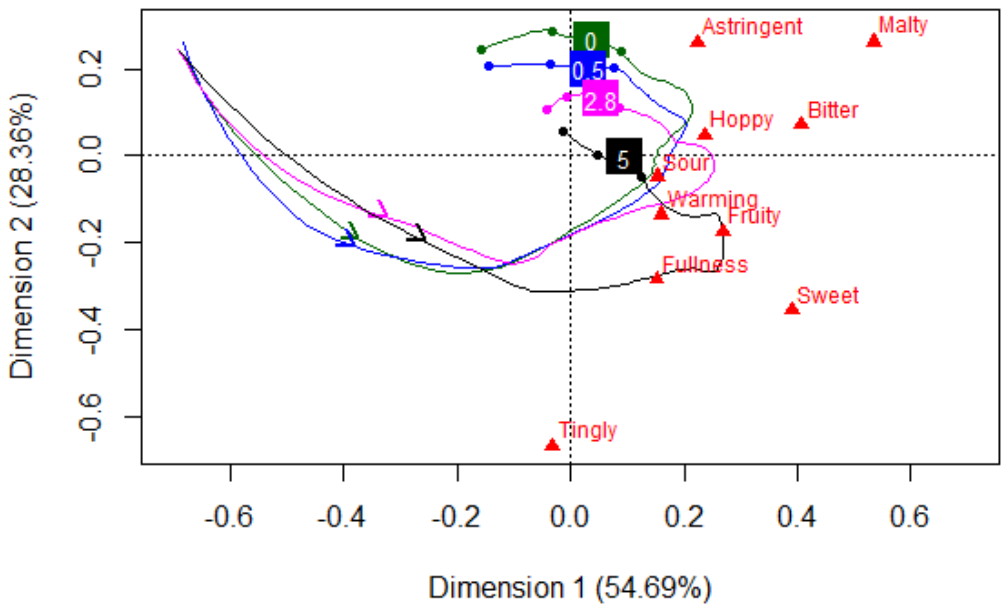


Figure 2. 3: *Principal components analysis (PCA) biplot of the TCATA citation of attribute data over the 60 s period for all beer samples. The arrow head > indicates swallow time (at 10 s) and shows the development of these attributes over the 60 s evaluation period. Beer sample trajectories are labelled with the ethanol concentration at the first 40 s of evaluation time. Time markers (dots) ● are positioned along the remainder of each of the trajectories at 5 s intervals to show progression of evaluation time.*

2.3.6 Relationships between Temporal Sensory Attributes (TCATA) and Temporal Liking (TL)

The random effects regression analyses highlighted the influence of the TCATA attributes on liking in each cluster. For C1, presence of tingly sensations exerted a significant positive influence on liking for all four samples (Table 2.6). For 0, 2.8 and 5% ethanol samples, having body also positively influenced liking. A sour note was a significant negative driver of liking ($p<0.001$) for all samples except for the 2.8% ethanol. Alcohol warming sensation was a negative driver of liking for both the 0 ($p=0.033$) and 0.5% ($p<0.0001$), becoming non-significant as the ethanol concentration increased. Presence of a fruity note was a negative driver of liking for the 0 ($p<0.0001$) and 2.8% ($p=0.047$), but positive for the 0.5 ($p<0.0001$) and 5% ($p<0.0001$) ethanol samples. Sweet was a significant negative driver of liking for the 0% ($p<0.0001$), yet when the ethanol concentration increased to 0.5% ($p=0.002$) and 5% ($p<0.0001$), this attribute became a positive driver of liking. Interestingly, bitter was a negative driver of liking for all samples ($p=0.048$ for 0% ethanol; $p<0.0001$ for 0.5% and 2.8% ethanol); however, at 5%, it became a significant positive driver of liking ($p=0.011$).

For C2 (Table 2.6), the significant positive drivers of liking for samples other than 5% ethanol were the presence of the attributes of malty ($p<0.0001$) and sweet for 0% ethanol ($p=0.003$) and 0.5 and 2.8% ethanol ($p<0.0001$). Other significant positive drivers of liking were presence of alcohol warming sensation for 0% and 5% ($p<0.001$), as well as 0.5% ethanol ($p=0.039$). The citation of the fruity attribute positively influenced liking in the 0% ethanol ($p=0.004$), 2.8% and 5% ethanol samples ($p<0.0001$).

Astringent ($p<0.0001$) and tingly ($p=0.034$) sensations were identified as significant positive drivers of liking for the 0% ethanol sample, but then significant negative drivers of liking for all the higher ethanol concentration samples ($p<0.0001$).

For C3 (Table 2.6), a sour note exerted a significant positive influence on liking for all beer samples (0% ethanol ($p=0.007$), 0.5% ethanol ($p<0.0001$), 2.8% ethanol ($p=0.014$) and 5% ethanol ($p<0.00001$). The citation of tingly positively influenced liking for all samples except the 2.8 % ethanol ($p<0.0001$). Sweet had a positive influence on liking for the 0.5% sample ($p<0.0001$); however, as the ethanol concentration increased to 5%, this negatively influenced liking ($p<0.0001$). A similar trend was observed with bitterness, exerting a positive influence on liking for the 0% ethanol ($p=0.002$) but the liking of 2.8 and 5% ethanol samples was negatively influenced by the presence of bitterness ($p<0.0001$).

Overall each cluster showed differences in terms of attributes which drove liking and disliking for all samples. C1 seemed to enjoy the mouthfeel attributes of tingly and fullness/body sensations at all ethanol concentrations, with the tastes of sweetness and bitterness seeming to be negative drivers of liking. C2 enjoyed malty and sweet attributes and disliked astringent and tingly sensations when ethanol concentration increased. C3 liked sour and tingly sensations and disliked bitterness as the ethanol concentration increased.

Table 2. 6: *z and associated p values from regression analysis denoting influence of TCATA attributes on temporal liking by cluster over consumption time. Black shading shows a significant negative driver of liking; grey shading shows a significant positive driver of liking*

Cluster 1								
	0% Ethanol		0.5% Ethanol		2.8% Ethanol		5% Ethanol	
Attribute	z-value	p-value	z-value	p-value	z-value	p-value	z-value	p-value
Malty	-5.30	**<0.0001**	1.77	0.077	4.51	**<0.0001**	-4.40	**<0.0001**
Astringent	-6.20	**<0.0001**	0.47	0.636	-6.13	**<0.0001**	0.55	0.580
Alcohol	-2.13	**0.033**	-4.14	**<0.0001**	0.48	0.634	0.35	0.728
Bitter	-1.98	**0.048**	-8.34	**<0.0001**	-6.33	**<0.0001**	2.55	**0.011**
Fruity	-4.77	**<0.0001**	5.10	**<0.0001**	-1.99	**0.047**	6.54	**<0.0001**
Body	3.15	**0.002**	-5.63	**<0.0001**	5.06	**<0.0001**	8.24	**<0.0001**
Sour	-11.00	**<0.0001**	-4.17	**<0.0001**	0.48	0.633	-6.57	**<0.0001**
Sweet	-4.89	**<0.0001**	3.15	**0.002**	1.51	0.131	5.20	**<0.0001**
Tingly	2.08	**0.037**	6.31	**<0.0001**	4.31	**<0.0001**	4.06	**<0.0001**
Cluster 2								
	0% Ethanol		0.5% Ethanol		2.8% Ethanol		5% Ethanol	
Attribute	z-value	p-value	z-value	p-value	z-value	p-value	z-value	p-value
Malty	6.37	**<0.0001**	5.17	**0.000**	8.91	**<0.0001**	0.90	0.369
Astringent	9.45	**<0.0001**	-2.47	**0.013**	-6.06	**0.000**	-7.17	**<0.0001**
Alcohol	6.38	**<0.0001**	2.06	**0.039**	-0.50	0.616	3.97	**<0.0001**
Bitter	0.14	0.892	1.50	0.134	3.76	**<0.0001**	0.16	0.871
Fruity	2.86	**0.004**	0.61	0.543	4.64	**0.000**	14.32	**<0.0001**
Body	0.09	0.926	-1.78	0.076	0.02	0.984	-4.93	**<0.0001**
Sour	-2.88	**0.004**	1.22	0.223	1.00	0.318	1.03	0.304
Sweet	2.94	**0.003**	7.92	**<0.0001**	4.59	**<0.0001**	-0.17	0.861
Tingly	2.12	**0.034**	-2.44	**0.015**	-5.57	**<0.0001**	-3.81	**<0.0001**
Cluster 3								
	0% Ethanol		0.5% Ethanol		2.8% Ethanol		5% Ethanol	

Attribute	z-value	p-value	z-value	p-value	z-value	p-value	z-value	p-value
Malty	-5.18	**<0.0001**	-4.30	**<0.0001**	0.95	0.342	-0.79	0.428
Astringent	-1.88	0.061	-2.61	**0.009**	3.88	**<0.0001**	-4.67	**<0.0001**
Alcohol	-0.32	0.749	-1.30	0.194	-0.88	0.380	-3.73	**<0.0001**
Bitter	3.13	**0.002**	1.44	0.150	-6.24	**<0.0001**	-5.17	**0.000**
Fruity	1.82	0.069	-1.69	0.091	3.97	**<0.0001**	0.31	0.760
Body	0.33	0.742	-0.02	0.986	9.24	**<0.0001**	1.18	0.239
Sour	2.69	**0.007**	3.52	**<0.0001**	2.46	**0.014**	4.31	**<0.0001**
Sweet	1.38	0.168	4.57	**<0.0001**	-5.15	**0.000**	-3.68	**<0.0001**
Tingly	15.88	**<0.0001**	5.28	**<0.0001**	1.12	0.261	7.36	**<0.0001**

2.4 Discussion

The market for low alcohol beer is increasing rapidly and so an understanding of the sensory properties that ethanol contributes to a beer is important. Here the impact of ethanol on the temporal sensory signature, as well as overall liking was investigated. Furthermore, whether a particular time point related to overall liking was explored, as were the temporal sensory drivers of liking.

The instrumental analysis confirmed ethanol concentrations of the beer samples to be in the regions of 0, 0.5, 2.8 and 5%, and showed significant differences among samples in terms of their pH and titratable acidity. As the ethanol concentration in the beer sample increased, the pH decreased and titratable acidity increased. The ranges in values measured were in accordance with typical values expected in beer (pH 4.0 ± 0.2) (Taylor, 1990). Despite ethanol concentration affecting changes in pH and TA, the differences were below the thresholds previously identified for sensory detection in wine (Amerine and Roessler, 1976) (0.02-0.05% for TA and 0.05 for pH). It is noted

that the medium in this latter study was wine and not beer and so these results cannot be applied directly, however no research has been done for beer. Therefore, it can be concluded that these parameters were unlikely to have contributed to a sensory difference across the beer samples.

2.4.1 The Influence of Ethanol Concentration on Liking

In the initial analysis of overall liking of the four beer samples, no significant differences were found. However, with the application of cluster analysis, three consumer clusters were identified and so understanding that there are individual differences within a population for beer liking in relation to ethanol content is key for the brewing industry in the development of new products (Guinard et al., 2001).

While differences in overall liking were found among clusters, no demographic predictors of cluster membership could be identified due to insufficient cell counts for the statistical analysis. The clusters were therefore likely to be a result of the differences in liking of the sensory profile of the samples brought about by the variation in ethanol concentration. C1 consumers preferred the high ethanol beer whilst C3 consumers preferred the low or no ethanol beer samples. C2 was composed of consumers who did not show any preference for the samples. Consumers within this cluster could be described as ‘enthusiasts’ as their overall liking for all samples was considerably higher than other clusters; a similar group was found in other products such as bread (Gellynck et al., 2009) and quinoa (Wu et al., 2017).

It is important to note that the number of consumers for C1 and C3 were too low to draw strong conclusions from and so the results for these

clusters can only be viewed as trends in the consumer data. Suggestions for future work would be to increase the number of consumers participating, to ensure stronger conclusions can be drawn from the data.

Previous studies have shown that liking is not a static measurement but rather a temporal event (Delarue and Loescher, 2004, Lee and Pangborn, 1986, Taylor and Pangborn, 1990, Veldhuizen et al., 2006). Consumers were able to perform the task of evaluating their liking over time, supporting previous research (Sudre et al., 2012, Thomas et al., 2015). The three consumer clusters created from the overall liking measurements reflected similar patterns of preference as the liking curves generated through TL. It should be noted that measuring OL straight after TL may have introduced some bias and could explain why the clusters followed similar patterns of liking for both liking measurements. Other research has shown similar results in orange lemonades, displaying relatively flat hedonic curves for temporal liking for the whole assessment procedure from ~2.5s to 30s (Veldhuizen, Wuister, et al., 2006). However, in a temporal study of liking of cheese, the most liked products overall were found to be liked significantly less at the beginning of evaluation, but this may be due to the change in product matrix through mastication (Thomas et al., 2015). Therefore a recommendation for further work would be to investigate the effects of multiple sips of beer on temporal liking as suggested in other literature (Jamieson and Wantling, 2017, Guinard et al., 1986).

In the current study, the liking of all clusters was shown to be significantly stable throughout the 60s evaluation period. Although the figures show some variability in liking for all products between 0-15s, further analysis

at earlier time points (5s and 8s) showed no significant differences in liking between time points ($p>0.05$). This may have been because liking by some consumers was registered as late as 26s into the evaluation period which may not reflect the normal experience for a consumer. Generally, temporal liking was found to be more discriminating than overall liking, with changes seen over the 60s consumption period. In C1, the temporal liking of the most liked sample (5% ethanol concentration) is maintained throughout evaluation, however for the least liked products the liking diminishes after swallowing. This is similar for C3, where the liking of the least liked sample (5% ethanol concentration) diminishes rapidly after swallowing.

2.4.2 Relating Overall Liking to Temporal Liking

The relationship between OL and TL was assessed to see at which time point consumers might base their overall liking. One of the main findings from this study was that OL and TL results gave consistent sample rankings for each cluster. In addition to this, TL evaluations were found to be fairly stable over time for all clusters, although they did highlight a drop in liking for some samples after swallowing. Only two studies to our knowledge (Sudre et al., 2012, Thomas et al., 2015) have linked time intensity of liking data or continuous liking with overall liking. In both of these studies, consumers registered their overall liking responses early in the consumption experience. In a study by Thomas et al. (2015) overall liking was recorded at 17s, with the total consumption experience being 36s, thus describing more of the first impression of the product rather than after swallowing/aftertaste of the product (Sudre et al., 2012, Thomas et al., 2015). Interestingly, in the current study, there was not a particular time that best related to liking. It appeared to be

dependent on ethanol concentration. As ethanol concentration increased in the beer samples, the time during the temporal evaluation that best related to overall liking shifted. For C1, as ethanol concentration increased from 0% to 5%, the time point that significantly related to overall liking decreased from 60s to 10s.The liking of the most liked sample (5%) in C1 was maintained throughout evaluation, with the lower ethanol concentration products diminishing in liking after swallowing. For C3 the overall liking did not significantly relate to temporal liking for any samples, apart from the 5% sample (at 20s), which was the most disliked product. This suggests that the highly liked and disliked products within each cluster related best to overall liking earlier on into evaluation. It could also have been due to familiarity of the beer, as the 5% sample is assumed to be closer to the consumers' expectations and so could be easier for them to evaluate. In addition, as consumers followed a strict procedure to drink the beer, this likely influenced their overall liking. Looking deeper into the data C1 (who preferred the 5% sample) and C3 (who disliked the 5% sample most) were found to perceive the ethanol related attribute of sweetness at 10s significantly more than C2 and so it could be deduced that these consumers either liked or disliked this respectively, which formed their overall liking score. Finally, the use of TL should be discussed based on the results of this study. TL for consumers appeared to be an easy task, but, not surprisingly, was longer and more cumbersome compared to OL. It gave stable results over time. TL evaluation may be well suited to foods where clear consumption periods can be defined (e.g mastication, swallow, aftertaste) or for drinks with strong aftertastes (e.g

bitter tea, coffee, wine) to understand the change in liking over these periods of consumption.

2.4.3 Influence of Ethanol on Sensory Attributes of Beer

2.4.3.1 TCATA

Overall, the TCATA curves showed a difference in temporal sensory profiles among all beer samples over time. As ethanol concentration in the beer sample increased, the citation of alcohol warming sensation increased, following results from other research in beer (Clark et al., 2011a). However, interestingly in the current study, alcohol warming sensation was only significantly cited more often during the ~55 to 60s time period in the 5% ethanol beer sample, reflecting its later presentation. This later presentation may have been due to the interaction effect of other factors within the beer, including the presence of carbon dioxide and hop acids, which have both been found to suppress warming sensation (Clark et al., 2011a).

CO_2 has also been found to interact with ethanol at lower ethanol concentrations (0, 2.25 and 4.5%) to modify warming sensation; this may explain why alcohol warming sensation was still cited at the 0% and 0.5% ethanol levels in the beer samples (Clark et al., 2011a). It has also been speculated that this could have been due to the irritation from the carbonic acid from the CO_2 (Dessirier et al., 2000, Simons et al., 1999).

The increase in ethanol concentration was also accompanied by the increased citation of other sensory attributes such as sweetness and fullness/body. Previous studies have found that ethanol enhances the perception of sweetness at ethanol concentrations between 0 and 24% (Clark et

al., 2011a, Martin and Pangborn, 1970). Ethanol (0.3-10%) stimulates sweet-best fibres due to taste-taste mechanisms, as well as activates nerve fibres sensitive to sugar which can be used to explain these differences among samples (Hellekant et al., 1997, Scinska et al., 2000). In terms of fullness/body, Langstaff et al. (1991) reported that the fullness of commercial beers was moderately correlated with alcohol content with correlation coefficients of 0.41 for density and 0.50 for viscosity.

No significant differences were found in the overall citation rates of flavour attributes malty, hoppy and fruity. Instrumental results using in-vivo atmospheric pressure chemical ionisation mass spectrometry (APCI-MS) by Clark et al. (2011b) found that as ethanol concentration increased from 0 to 4.5% the in-breath release of ethyl acetate, isoamyl alcohol and phenylethyl alcohol increased. This may suggest an expected increase in citation of related sensory attributes, however this was not the case here, and hence if volatile release was higher in the higher ethanol samples this was not perceivable. The differing results between this study and Clark et al. (2011b) could have been due to the volatile compounds measured and their correlated sensory attributes (Conner et al., 1998).

No significant differences were found in the current study in the overall citation rates of astringency, but when looking at the temporal evaluation of this attribute the lower alcohol samples were found to be significantly more astringent towards the end of consumption time, with this attribute being temporally negatively correlated with PC2.

The onset of attributes also differed in that some attributes were cited more frequently earlier in the evaluation time, while others were delayed and thus were cited later in the evaluation time. For all beer samples, tingly sensation was one of the first attributes to appear. Delayed onset attributes which appeared after swallowing included malty flavour, bitterness and hoppy flavour. Work by Missbach et al. (2017) showed similar results with worty off-flavour being most pronounced between 0 and 30s, with the dominance of malty flavour increasing after swallowing. Bitterness was also found to dominate the flavour profile after swallowing. A study by Vázquez-Araújo et al. (2013) showed a similar time to maximum intensity of both hoppy flavour and bitter taste in commercial lagers. Bitterness was also found to be the attribute which lingered longer, and estery/fruity notes were found to abate first (Vázquez-Araújo et al., 2013).

2.4.3.2 Influence of Temporal Sensory Attributes on TL

Acceptance of the beer samples was also contextualized by an examination of the TCATA attributes. Thomas et al. (2015) found that the dominance of attributes plays a role in consumer liking, however the drivers of liking are mainly through the synergy of several components. The present study supported this earlier finding, showing that all attributes (and not just dominant attributes) were related to ethanol concentration and liking within the three different clusters of consumers.

C1 (who preferred the 5% sample) were found to like tingly and fullness/body attributes, which are both linked to a higher ethanol concentration. In addition, alcohol warming sensation was a significant driver of disliking at the lower concentrations, with the consumers also disliking

sourness mostly in the 0% beer. Alcohol has been reported to suppress sourness due to the decrease in the physiological response of the chorda tympani nerve in the presence of a sour stimulus (Martin and Pangborn, 1970). The consumers in C1 in the present study also disliked bitterness until the ethanol concentration reached 5%, when it became a positive driver of liking. Ethanol concentration has been found to have an additive effect on bitter sensation as it intensifies flavour perception (Martin and Pangborn, 1970, Meillon et al., 2010, Missbach et al., 2017) thus the consumers within this cluster may have perceived this at the higher concentration.

C2 (who liked all samples) liked malty flavour, sweet taste and alcohol warming sensation. Interestingly a study by Porretta and Donadini (2008) showed similar results, with conclusions being drawn that overall flavour preference was highest for a malty flavour beer, which reflects the fact that this was the largest beer consumer cluster. Consumers within C2 disliked astringent and tingly sensations when the ethanol concentration was increased to 0.5%, and ethanol has been found to enhance both of these sensations.

C3 (who disliked the 5% sample most) enjoyed sourness and tingly sensations and disliked alcohol, bitter and sweet attributes perceived within the 5% sample. All these attributes can be related to the added ethanol within the beer and the interactions between the components impacting sensory perception (Clark et al., 2011a). Conclusions can be drawn from this study that attributes are not only drivers of liking or disliking depending on the ethanol concentrations of beer samples, but that these vary depending on the consumers, as was evident from the clustering. One hypothesis for this is that at different concentrations of ethanol different attributes are enhanced or

masked which drive liking/dislike in the different clusters differentially. It is important to note that the balance of the overall profile of attributes is just as important as the particular attributes themselves and so this needs to be considered when developing a new low alcohol beer, to form a favourable product; although this may only be a favourable product to some consumers within a population. It is recognised that one limitation in this study is that the beers were not fully optimised as would happen commercially when changing the ethanol concentration. This may also have had a difference in the integration of the flavour compared to when the beer is brewed to a certain alcohol percentage. The use of dealcoholisation apparatus to develop a base non-alcohol beer which can be adjusted for its chemical composition and to produce samples only varying in ethanol content, may offer improved insights into the effects of ethanol concentration. In addition to this, this study only looked into the effect of ethanol concentration in the context of lager and therefore this does not necessarily apply to other beer styles, which would be an interesting area for future research.

Many papers have looked at combining overall liking data with TCATA, TDS and CATA results (Ares et al., 2017, Thomas et al., 2017, Thomas et al., 2015), however to the authors' knowledge this is the first paper to combine TCATA data with temporal liking. However the fact that only ten attributes were included could be seen as a limitation as others characteristics may be important but were not included on the list. Using a temporal measure of liking enabled additional insights into which aspect of the product drove liking via the combination of TL and TCATA results and/or at what time of the consumption process.

2.5 Conclusions

This study evaluated the influence of ethanol on the temporal perception of beer including both the perception of liking and sensory attributes, as well as identified critical attributes that drive consumer acceptance. Overall, it showed that consumers can be clustered to show their liking and disliking of beer samples containing different ethanol levels, including a cluster that liked low/no alcohol beer products similarly to standard beers. A study with larger numbers of consumers would help confirm this.

This study also reported the relationship between temporal liking and overall liking to understand particular time points in products where consumers judge their overall liking, with results showing this was dependent upon the consumer, as well as the ethanol content of the beer sample. In the higher ethanol samples, liking was determined more rapidly compared to the lower alcohol samples. In addition, differences in sensory attributes among beer samples with different ethanol concentrations were described, with a 5% beer having significantly more sweetness, fullness/body and alcohol warming sensation, highlighting the importance and role of ethanol within beer.

This research is important for the brewing industry as it shows the overall sensory experience during consumption of a beer. It provides valuable insight into a broad range of sensory attributes which are altered when ethanol is modified in beer, and highlights which attributes should be targeted by manufacturers when developing new low alcohol products. A new technique giving greater insight into liking was also described to link temporal liking with TCATA results to understand the drivers of liking at certain time points across different products.

3 Sensory evaluation, aroma release and molecular hydrodynamics: a combined approach towards understanding the lost functionality of ethanol in non-alcoholic beer

Preliminary thoughts Chapter 3:

The effect of ethanol concentration perceived by naïve beer consumers on the sensorial qualities of NAB was reported in Chapter 2, and showed that ethanol enhanced perceptions of sweetness, fullness/body and alcohol warming sensation within a beer matrix. However, it is unclear if the sensorial differences between beers were due to physicochemical changes within the matrix or due to multimodal flavour perception. This is of particular importance considering ethanol enhanced the perception of sweetness and alcohol warming sensation and these were key drivers of liking for a large cluster of consumers (C2).

Previous studies have reported the influence of ethanol concentration on changes in volatile aroma release in ethanol/water solutions, wine and model beer solutions (reported in section 1.4.). These have been measured

using static, dynamic and in-vivo flavour analysis techniques, although some disagreement has been shown amongst the results of these methods. The threshold for changes in volatile partitioning have been cited as around 17% v/v, however recent research has suggested that this may in fact be lower (reported in section 1.4.3). Further research to understand physicochemical changes at lower ethanol concentrations relevant to beer is therefore required.

The combination of analytical and sensory evaluation techniques to understand both the physicochemical and sensorial changes that occur with the removal of ethanol in the production of NAB, so that improvements can be made, have yet to be reported. Commonly, static headspace techniques are employed, which can be related to orthonasal sensory perception, however these fail to take into account changes occurring during retronasal consumption. Previous research has also shown that the non-volatile matrix, such as proteins and carbohydrates, affects headspace partitioning. With this being the case, it is important to explore differences in static aroma partitioning between different beer styles such as lager and stout.

Therefore in the following study, differences between orthonasal and in-mouth flavour sensory perception were explored using sensory techniques, instrumental methods and the addition of saliva into the matrix as a novel technique to analyse the impact of ethanol.

This chapter was submitted as a paper to Scientific Reports in June 2020 and was accepted for publication in November 2020:

Ramsey, I., Dinu, V., Linforth. R., Yakubov, G., Harding, S., Yang, Q., Ford, R., and Fisk, I. (2020) Sensory evaluation, aroma release and molecular hydrodynamics: a combined approach in understanding the lost functionality of ethanol in non-alcoholic beer. *Scientific Reports*, 10, 20855.

doi: https://doi.org/10.1038/s41598-020-77697-5

Sensory evaluation, aroma release and molecular hydrodynamics: a combined approach towards understanding the lost functionality of ethanol in non-alcoholic beer

Imogen RAMSEY[ab], Vlad DINU[bc], Rob LINFORTH[b], Gleb E. YAKUBOV[cd], Stephen E. HARDING[c], Qian YANG[a], Rebecca FORD[a] and Ian FISK[b]*

[a]Sensory Science Centre, Division of Food, Nutrition and Dietetics, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, United Kingdom

[b]Food Flavour Laboratory, Division of Food, Nutrition and Dietetics, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, United Kingdom

[c]National Centre for Macromolecular Hydrodynamics, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, United Kingdom

[d]Biomaterials Laboratory, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, United Kingdom

Highlights:

- Consumers reported no significant change in orthonasal properties, but in-mouth flavour results showed 0% ABV alternatives are significantly more malty, with sensory scores for fruity, sweet and fullness/body and alcohol warming sensation decreasing.
- Ethanol reduces the static aroma partitioning of flavour compounds in 5% lager and stout compared to the 0% ABV alternatives.
- The *in vit*ro analysis revealed an ethanol* saliva interaction effect on the headspace concentration of hydrophobic compounds.
- Salivary α-amylase denatures with the increase of ethanol concentration, leading to unfolded structure with less hydrophobic pockets
- At 5% ABV ethanol, changes in protein conformation correlate with the increasing headspace concentration for hydrophobic compounds and decreasing for hydrophilic ones.

Abstract

Consumer sensory evaluation, static aroma partitioning analysis and biophysical protein analysis were used to investigate the effect of ethanol on the release and perception of flavour in beer (lager and stout) at different ethanol levels (0 and 5% ABV). Consumer study results showed no significant differences in orthonasal perception, yet in-mouth flavour results showed that 0% lager was perceived as maltier with reduced fruitiness, sweetness, fullness/body and alcohol warming sensation ($p < 0.05$). Whilst ethanol alone decreases the static aroma partitioning regardless of Log*P*, the presence of α-amylase selectively reduces the headspace concentration of hydrophobic compounds. It was found that ethanol has a subtle inhibitory effect on the binding of hydrophobic compounds to α-amylase, thereby increasing their headspace concentration in the 5% ABV as compared to the 0% beers. This synergistic ethanol*saliva effect is attributed to the changes in the conformation of α-amylase due to ethanol-induced denaturation. It is hypothesised that the partially unfolded protein structures have a lower number of hydrophobic pockets, leading to a lower capacity to entrap hydrophobic aroma compounds. This supports the hypothesis that ethanol*saliva interactions directly impact the sensory and flavour properties of beer, which would provide a basis for further investigations in reformulation of 0% ABV drinks.

3.1 Introduction

Beer is one of the most widely consumed beverages around the world, with production increasing by 3 million litres from 2014 to 2019 (Euromonitor, 2019b). However, sales of standard alcohol beer in the UK have been steadily decreasing (Mintel, 2019c). One of the key factors behind this trend is consumers' desire to limit their alcohol consumption in order to reduce the risks associated with alcohol-related diseases and other considerations (Mintel, 2017a). The worldwide non-alcoholic beer (NAB) market is predicted to increase in value to $25 billion by 2024, as consumers begin to express more interest in lower alcohol counterparts (Verma and Rawat, 2018). Therefore, there has been increased development within the NAB sector, with research focusing on understanding the physicochemical properties and sensory attributes of the product matrix in order to improve the quality and experience of the non-alcoholic product.

The composition of the food or beverage plays a key role in the release of flavour compounds (Piornos et al., 2019, Ployon et al., 2017). These can include the chemical characteristics of volatile compounds (volatility, polarity, and hydrophobicity) as well as the physicochemical properties (chemical composition, physical properties, texture and viscosity). Beer matrix components can be broadly classified into two groups; volatiles which include a wide range of compounds such as aliphatic and aromatic alcohols, esters, acids, carbonyl compounds, terpenes; and non-volatiles which include the ethanol and larger macromolecules such as polysaccharides, proteins and nucleic acids, as well as inorganic salts, sugars, amino acids, nucleotides, polyphenols and hop resins (Castro and Ross, 2013). All components found in

beer play an important part in the final product matrix, but little is known about the effect these components have on flavour release during consumption. In order to understand the functionality of ethanol, further insights into its' effects on the organoleptic profile are required to develop low/no alcohol beverages which have the same desirable sensory attributes and high consumer acceptance as standard alcoholic drinks.

To tackle some of the challenges, previous work has looked at the impact of ethanol on the sensory properties of beer. Clark et al. (2011a) used a trained sensory panel to identify differences between beers with different ethanol concentrations (0, 2.25 and 4.5% ABV). However, no differences were found in terms of separate aroma and flavour attributes, but an enhanced warming mouthfeel, sweetness and complexity of flavour was observed (Clark et al., 2011a). In another study Missbach et al. (2017) found that malty was the most pronounced attribute in an alcohol-free beer after swallowing the sample. Perpete and Collin (2000) used purge and trap and thermal desorption cold trap extraction to measure aldehydes in beers with different ethanol concentrations. They found that increasing the ethanol concentration of a beer from 0 to 5% showed increased retention of aldehydes, such as 2-methylbutanal and 3-methylbutanal, which are responsible for the 'worty' off-flavours in NAB (Perpete and Collin, 2000).

Ethanol clearly has a substantial effect on the overall sensory properties of beer. Consequently, to further scientific understanding of these perceptual changes, researchers have looked at the impact of ethanol on headspace partitioning of volatiles using classical headspace techniques such as solid phase micro extraction gas chromatography mass spectrometry (SPME-GC-

MS). These studies mostly found that as ethanol concentration increases there is a decrease in headspace concentration, with ethanol altering the polarity of the product matrix and increasing the solubility of aroma compounds (Aznar et al., 2004, Boelrijk et al., 2003, Conner et al., 1998, Escalona et al., 1999, Perpete and Collin, 2000, Tsachaki et al., 2008). These static headspace techniques are highly useful in the study of aroma interactions within the product, as they can be used to find subtle differences, which may be underestimated by dynamic methods (Mitropoulou et al., 2011). Though conversely, static headspace measurements alone fail to take into account other conditions such as air sweeping, saliva mixing, mastication and temperature changes, which occur during consumption (Clark et al., 2011b). To alleviate these shortcomings of static headspace analysis and capture the real life dynamic aspects associated with oral processing, researchers are developing and beginning to apply novel methods.

One of the ways suggested to understand some of the dynamic changes in flavour release is through the analysis of the bolus, which ultimately plays a role in the perception and release of flavour. This is achieved through the inclusion of saliva, or its components, which are known to have a significant effect on the retronasal release through interactions with aroma molecules (Yakubov et al., 2014). Saliva is a complex mixture made up of water (97 wt%) and a range of salivary proteins and electrolytes. Salivary α-amylase, mucins and proline rich proteins (PRP's) are the most abundant of the salivary proteins, contributing to over 90 % to the entire salivary protein content (Humphrey and Williamson, 2001). These proteins and glycoproteins are responsible for the key physicochemical properties of the saliva, such as

viscoelasticity, lubrication, control of Ca^{2+} super saturation and buffering capacity (Gittings et al., 2015, Yakubov, 2014). Past research recognised some of the fundamental roles of saliva, in addition to the ingredients used in the formulation of food. Therefore, scientists began to analyse its effects on the generation of flavour, although studies generally focused on studying the release and partitioning of volatile aroma compounds in single component systems, such as solutions of sugars, salts or individual food proteins (Friel and Taylor, 2001, Jouenne and Crouzet, 2000).

During food oral processing, the interactions between salivary proteins and flavour molecules in the bolus are proposed to have a significant role in flavour perception. Hence, recent studies are now beginning to characterise some of the more complex interactions underpinning the partitioning of aroma compounds from the bolus during the oral processing pathway (Ayed et al., 2018, Boehm et al., 2019, Boehm et al., 2020, Dinu et al., 2018). However, like most studies on food, the majority of research on beer examines the influence of ethanol on the partitioning of individual aroma compounds in water/ethanol solutions (Ammari and Schroen, 2019, Aprea et al., 2007), although one has examined flavour release in a model beer (Clark et al., 2011b). Furthermore, to the best of the author's knowledge no studies to date have investigated flavour interactions during oral processing in a real beer matrix and, in particular, a non-alcoholic one. Addressing this problem is timely due to the rise of NAB sales, and the need for brewers to improve palatability and acceptability in this sector.

Our work aims to support some of these challenges by using a novel combined approach, in order to understand the differences in the

physiochemical dynamics of aroma release and flavour perception between a 0% and 5% ABV beer. The objectives of this study were therefore to explain the orthonasal and in-mouth flavour differences in consumer perception of standard and NAB. This was achieved by quantifying the effect of the ethanol*saliva interplay on flavour release through consumer sensory evaluation, headspace analysis of aroma compounds and macromolecular hydrodynamics.

3.2 Results

3.2.1 Consumer Sensory Evaluation – Orthonasal Aroma vs In-Mouth Flavour Perception

For the consumer analysis, the lager style beer was chosen to understand sensorial differences between the orthonasal aroma and in-mouth flavour properties. For the orthonasal analysis, citation rates for the six aroma attributes provided in the lexicon did not reveal any significant differences, apart from minor changes in fruity aroma (Table 3.1).

Table 3. 1: *Citation rates of attributes in the description of orthonasal aroma of beer samples. A different letter in a row represents a significant difference in citation between samples as by Cochran's Q Analysis and Bonferroni multiple comparisons ($p<0.05$).*

Aroma Attribute	***p-value***	***0%***	***5%***
Fruity	***0.042***	*0.327 (a)*	*0.455 (a)*
Malty	*0.622*	*0.564*	*0.535*
Hoppy	*1.000*	*0.297*	*0.297*
Stale	*0.303*	*0.356*	*0.41*
Cooked Vegetable	*0.527*	*0.475*	*0.436*
Alcohol	*0.178*	*0.218*	*0.287*

However, whilst fruity aroma reached significance at $p = 0.042$ it did not show a significant difference in grouping after post-hoc test. In the next part of the study, consumers were asked to consume the lager samples and rate subsequent changes in flavour, taste and mouthfeel attributes over a 60 second time period. This time, the average proportion of citation data from in-mouth Temporal Check-All-That-Apply (TCATA) analysis showed significant differences between ethanol samples with respect to flavour, taste and mouthfeel attributes ($p < 0.05$) (Figure 3.1). The citation rates for flavour perception of 0% lager were found to be more malty and less fruity, with no significant changes in hoppy flavours. In terms of taste, the 0% lager appeared to be significantly less sweet with no significant changes in bitter and sour attributes. In terms of

mouthfeel, significant differences were identified for body and alcohol warming sensation, which scored much lower values for 0% lager (p = <0.0001 for both attributes). Similar results have been reported previously (Clark et al., 2011a, Langstaff et al., 1991, Missbach et al., 2017), suggesting that there are changes in the flavour profile occurring during the short amount of time upon consumption. Therefore, in order to elucidate some of the interaction mechanisms underpinning the perception of flavour in regular 5% ABV beer, the *in vitro* static partitioning of aroma compounds in a 0% and 5% ABV beer was investigated. Here, a stout style of beer was also included in order to examine any changes attributed to differences in beer matrix.

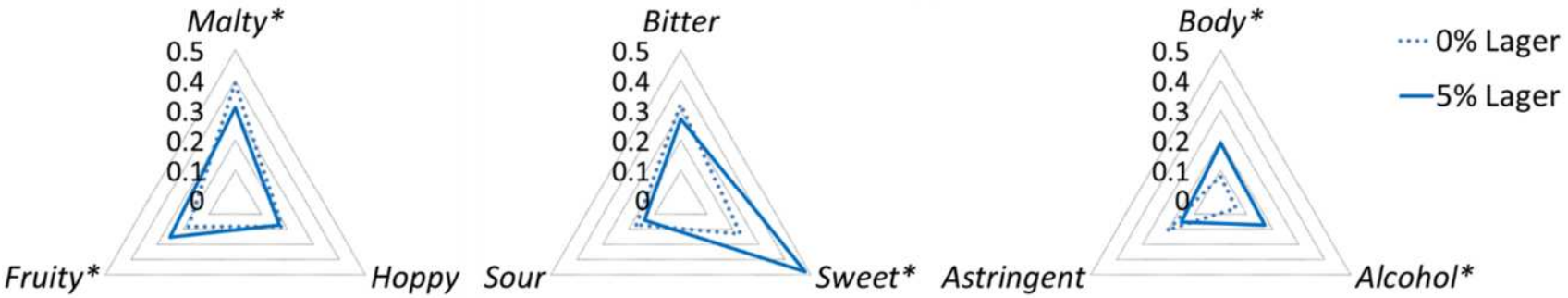


Figure 3. 1: *Average proportion of citation for consumer panel using in-mouth TCATA sensory attributes divided into flavour, taste and mouthfeel, showing significant differences between samples (Tukey's HSD Test ($p < 0.05$)*).*

3.2.2 Ethanol Effect on the Static Aroma Partitioning of Aroma Compounds in Different Beer Styles

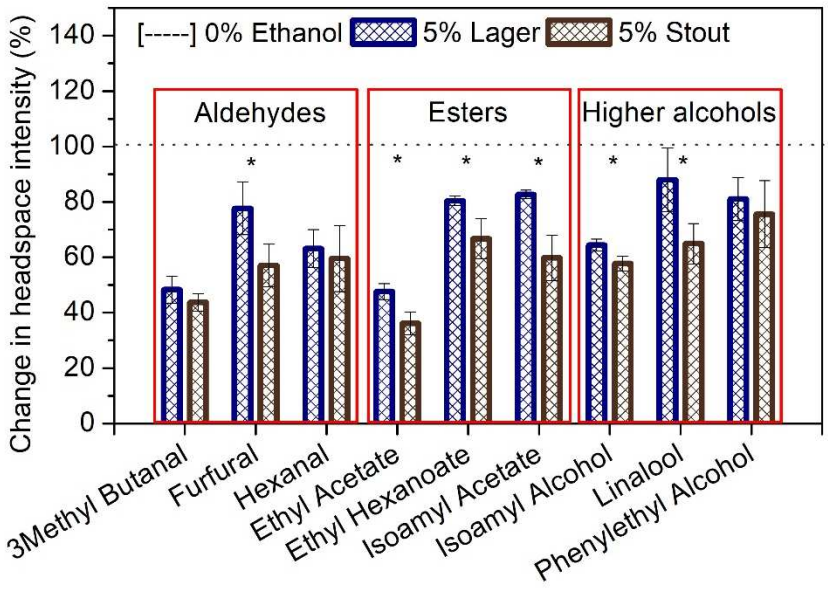


Figure 3. 2: *Effect of ethanol on in vitro static aroma partitioning in lager and stout style beers by GC-MS. Data grouped into aldehydes, esters and higher alcohols. Plot shown as relative changes normalised to 0% lager and 0% stout (data given as mean ± SE, n=4). * shows significance ($p<0.05$) in volatile partitioning between different beer styles.*

Firstly, the effect of ethanol on the partitioning of aroma compounds was examined by GC-MS analysis, in both lager and stout style beers (Figure 3.2). Aroma partitioning results were in agreement with the published literature, which revealed significantly lower intensities ($p < 0.05$) in the presence of ethanol (5%) as opposed to the controls (0%), in both beer styles. All compounds except furfural were significantly lower in the presence of ethanol in the lager ($p < 0.05$) although phenylethyl alcohol was not significant in the stout (see

Appendix Table 1). Similar effects of ethanol have been reported when measuring static headspace in model solutions (Conner et al., 1998, Perpete and Collin, 2000). These studies suggested that this is due to ethanol increasing the solubility of aroma compounds in the beer and therefore reducing their partition coefficient and concentration in the headspace (Aznar et al., 2004, Escalona et al., 1999, Tsachaki et al., 2008).

Matrix dependant effects were also observed between the two different beer styles; with the aroma concentration in the stout headspace significantly lower than in the lager for most compounds (Figure 3.2). This suggests that the flavour matrix interaction is affected by the presence of ethanol and/or ethanol changes the properties of the matrix. In the current study this is attributed to the stout having higher amounts of carbohydrates and proteins present in the sample (6.7 g/100mL carbohydrates, 3.1 g/100mL of which sugars, 0.6g /100mL protein – information provided on product label), compared to the lager (5.6 g/100 mL carbohydrates, 1.7 g/100mL of which sugars, 0.3 g/100mL protein – information provided on product label) suggested to physically lower the release of volatiles in the stout.

3.2.3 α-Amylase-Ethanol Interactions in Beer

3.2.3.1 GC-MS Results

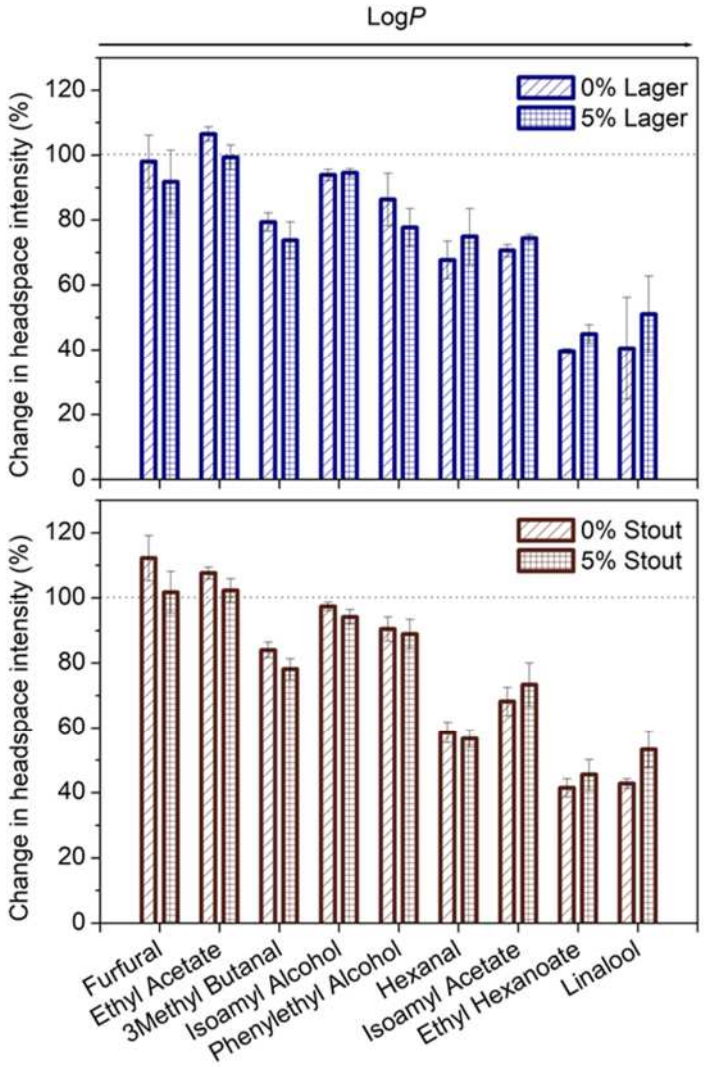


Figure 3. 3: *Changes in the static aroma partitioning of 0% and 5% beer in the presence of α-amylase by GC-MS. Aroma compounds listed in accordance with LogP coefficient to illustrate the effect of compound hydrophobicity on the aroma-protein interactions. Plot shown as relative changes normalised to controls (respective buffer samples shown as dotted line) for lager and stout (data given as mean ± SE, n=4).*

Salivary α-amylase is the most abundant salivary protein, comprising of over 60% of the total protein concentration in stimulated saliva (Mandel et al., 2010). To investigate the effect of saliva mixing and bolus formation during oral processing and its effects on the in-mouth flavour perception pathway, the effects were evaluated in the presence and absence of α-amylase. It was found that the presence of the salivary enzyme led to a decrease in the aroma release, with significant effects for the more hydrophobic compounds (Figure 3.3). Changes are shown relative to their respective controls (buffer samples, before α-amylase addition), corrected for volume to eliminate dilution effect. Of the aroma compounds measured, ethyl acetate, 3-methylbutanal, isoamyl alcohol, hexanal and isoamyl acetate showed significant differences in terms of post-hoc groupings in the lager. Furfural, ethyl acetate, 3-methylbutanal, isoamyl alcohol and isoamyl acetate were significant in the stout ($p < 0.05$) (see Appendix Table 1).

Individual differences in the aroma profile for the lager and stout beers were further analysed in a radar plot as a function of hydrophobicity in order to understand the effect of the salivary protein during the consumption of 0% and 5% ABV beers (Figure 3.4). The observed log*P* dependant effects were twofold: the increase of the relative proportion of the hydrophobic aroma compounds in the 5% beers and the decrease of the relative concentration in the 0% beers. Conversely, this meant that the presence of α-amylase led to a higher relative intensity of hydrophilic aroma compounds for the 0% ABV beer, although compounds such as hexanal and phenylethyl alcohol did not appear to follow this trend. A correlation plot is further given as Appendix Figure 1. In addition, this effect was corroborated in both beer styles, acting as a type of validation of

the effect, helping to provide some clues about the perception differences of NAB, observed via the in-mouth flavour evaluation in Figure 3.1.

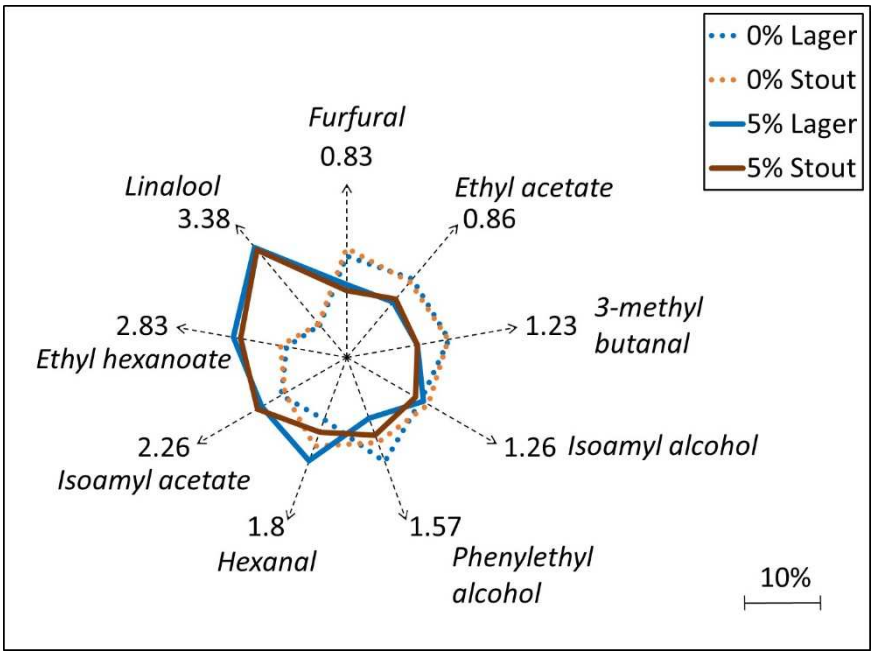


Figure 3. 4: *Radar plot analysis of the effect of ethanol on aroma – α-amylase interactions in 0% vs. 5% for different beer styles. Results given as a function of hydrophobicity (LogP) showing a lower proportion of hydrophilic compounds and higher proportion of hydrophobic aroma compounds released in the 5% lager and stout.*

3.2.3.2 Hydrodynamic Analysis of α-amylase at different ethanol levels

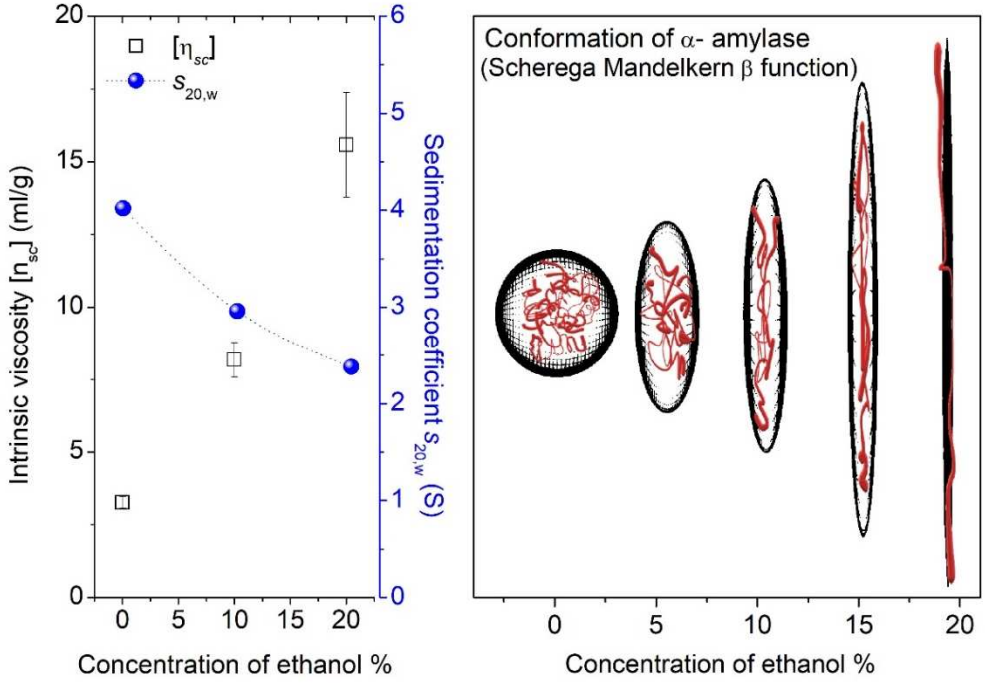


Figure 3. 5: *Hydrodynamic analysis of α-amylase as a function of ethanol. Results show the values for the intrinsic viscosity [η_{sc}] and sedimentation coefficient $s_{20,w}$ (S), used to illustrate changes in the conformation of α-amylase at higher ethanol concentrations. Prolate ellipsoids were generated in ELLIPS1 using the β-function of the Scherega-Mandelkern equation.*

To examine the effects of ethanol on saliva, the hydrodynamic stability of α-amylase was measured in the presence of different concentrations of ethanol. Ethanol was found to have an effect on intrinsic viscosity and sedimentation coefficient of α-amylase. At higher ethanol concentrations, the sedimentation coefficient of α-amylase decreased while the intrinsic viscosity

increased (Figure 3.5). Combining the two sets of data, a rapid method was employed to determine the gross conformation of the enzyme based on the classical Scherega-Mandelkern relationship (Scheraga and Mandelkern, 1953). This was achieved by computing the β term in equation 3.2, from the accurate measurements of its hydrodynamic parameters: sedimentation coefficient *s*, intrinsic viscosity [n] and molar mass *M*, by ensuring each series of *s*, [n] and *M* measurements are made in the same ethanol/water solutions. By using the program ELLIPS (García de la Torre and Harding, 2013), the calculated β function values were converted to prolate ellipsoid representations given by their consequent changes in axial ratios (Figure 3.5). Since the molar mass of α-amylase is constant, these changes in the anisotropy of α-amylase are suggested to arise from the uncoiling of the polypeptide chain as a result of ethanol denaturation. This effect is essentially a common type of alcohol denaturation where ethanol disrupts the hydrogen bonding of the protein structure, instead forming new hydrogen bonds with the polypeptide chains (Brandts and Hunt, 1967, Nikolaidis and Moschakis, 2018, van Koningsveld et al., 2002). Although, these effects may differ as a function of protein diversity and heterogeneity in saliva, as well as surface glycosylation, we suggest that the use of α-amylase as a test molecule highlights the generic mechanics and can markedly contribute to the physiological changes, given α-amylase abundance in saliva.

3.3 Discussion

When it comes to analysing the differences in the sensory profile of non-alcoholic beers (NABs), smelling the samples alone (orthonasal evaluation) is not enough to discriminate between aroma attributes, suggesting that ethanol itself has no significant effect on the aroma perception. However, when ingested (in-mouth flavour perception), significant differences were determined which showed the 0% beer to be maltier, with reduced fruitiness, sweetness, fullness/body and alcohol warming sensation. This was in full agreement with previous reports for NAB, suggesting that saliva is an important factor in sensory perception. This data indicates that product reformulation cannot be based solely on the physiochemical analysis of the product. Similar results were also found by Peltz and Shellhammer (2017), with ethanol concentration having little effect on the orthonasal detection for specific hop compounds in beer. Missbach et al. (2017) also agreed with these findings, in which they showed that malty is the most pronounced attribute in alcohol-free beer after swallowing. Likewise, others found that NABs have increased aldehyde retention of more hydrophilic compounds such as 2-methyl and 3-methylbutanal and methional, thus increasing worty-off flavours (Perpete and Collin, 2000). The same effect was shown in the headspace and sensory analysis, although consumers signified this change through the attribute ‘malty’. Ethanol has also been found to enhance sweetness, alcohol warming sensation (Clark et al., 2011a) and fullness/body (Langstaff et al., 1991) confirming the results found in this study. Therefore, it was indicated that ethanol has a significant effect on the in-mouth flavour perception of beer. Other reasons for the differences in non-volatile attributes could also be explained by multimodal flavour perception, as ethanol is

perceived by gustatory, olfactory and trigeminal modalities (Taylor et al., 2010). It should also be noted here, that taste-aroma interactions occur within the mouth, which could have been responsible for the differences discovered between orthonasal and in-mouth results. Previous research on bioactive food ingredients has however shown that volatile related attributes appear to be down to the interactions with salivary proteins (Ayed et al., 2018, Boehm et al., 2019, Dinu et al., 2018).

Therefore in order to examine the ethanol*saliva hypothesis in more detail and provide a mechanism-based understanding, a series of *in vitro* experiments were designed to evaluate the effect of ethanol, beer matrix and effect of salivary proteins, which are discussed further. Key aroma compounds that impart the recognised and desirable flavour of beer (aldehydes, esters and higher alcohols (Briggs et al., 2004)) were chosen to understand differences in the aroma release of beer. At 5% ABV, the headspace intensity of aroma compounds was lower than in the 0% ABV for both beer styles due to the solubility of aroma compounds in ethanol, reducing their concentration in the headspace (Aznar et al., 2004, Escalona et al., 1999, Tsachaki et al., 2008). All compounds were affected in a similar way by the presence of ethanol, and the rate at which they were released could not be explained by their physicochemical properties. Previous research has shown that hydrophobicity plays a role, with more hydrophobic compounds showing a significant decrease in headspace concentration with increasing ethanol concentration (Aznar et al., 2004, Boothroyd et al., 2012, Escalona et al., 1999, Tsachaki et al., 2008). However, both of these studies used APCI-MS in model solutions as well as much higher

alcohol concentrations, therefore this theory may not apply to a complex matrix system such as beer.

The effect of product matrix was analysed by comparing the aroma release from lager and stout, the latter having a higher macromolecular content. As a result, aroma release in the stout was lower in comparison to the lager. Previous research by Castro and Ross (2013) has shown that the non-volatile matrix affects the headspace partitioning, as well as the sensory perception of volatile compounds in a model beer due to a physical suppression effect. Other research has also shown that an increased proportion of macromolecules in solution affects the rate of diffusion of aroma compounds, thereby leading to a lower aroma release (Dinu et al., 2019b, Guichard, 2002, Jones et al., 2008, Philippe et al., 2003).

The presence of α-amylase in the GC-MS static aroma partitioning analysis showed that the rate at which these compounds changed was dependent on compound hydrophobicity, especially pronounced for higher log*P* compounds such as ethyl hexanoate and linalool. It is suggested that this effect is due to hydrophobic interactions between α-amylase and the aroma compounds. Previous research has confirmed these types of hydrophobic interactions, with an increase in the retention of aroma compounds by components found in saliva (mucin and α-amylase) (Muñoz-González et al., 2014a, Pagès-Hélary et al., 2014, van Ruth et al., 2001) as these aroma compounds are known to bind to salivary proteins and other macromolecules. Muñoz-González et al. (2019) also found that the oral release of ethyl hexanoate and isoamyl acetate was not affected by variations in ethanol content in wine directly. These researchers used an intra-oral SPME procedure where they

captured volatiles on a SPME fibre immediately after panellists had rinsed and expectorated wine samples (Muñoz-González et al., 2019). The change discovered in the present study however, was found to be minimal and may not be the only cause for the changes in orthonasal and in-mouth flavour sensory measurements. Therefore the authors propose that future work should measure these dynamic changes in-vivo, by using APCI-MS or BIOVOC breath samplers (Markes International, UK) to rule out other principles such as taste-aroma interactions that could be causing these changes. This consequently shows the impact of using *in-vivo* or *ex-vivo* techniques that factor in more real-world consumption dynamics, such as interaction with saliva to form a bolus and its subsequent effects on taste and aroma release.

Although a remarkable effect, the effect of changing from hydrophilic malty to hydrophobic fruity flavours with the addition of ethanol is not a new finding, which has been confirmed by previous research by Boothroyd et al. (2012). They observed that during the dilution of spirits to lower ABVs for nosing, some molecules are more likely to go through structural changes and form agglomerates, which capture hydrophobic aroma compounds. They discussed that this lowers their release into the headspace and changes the aroma of lower ethanol content solutions towards more polar, hydrophilic compounds. Current findings are conceptually similar to some observations reported in the previous work (Boothroyd et al., 2012), but in addition they provide a deeper insight into the role of salivary proteins, subjected to a certain degree of ethanol denaturation. This hypothesis was probed through molecular hydrodynamics by analysing the anisotropy of the enzyme, in the presence of different ethanol concentrations. Results found that higher ethanol concentration increased its

intrinsic viscosity and decreased its sedimentation coefficient. Through computational analysis, it was shown that the conformation of α-amylase changed from globular to elongated structures, suggested to arise from the uncoiling of the polypeptide chain as a result of ethanol denaturation. This common type of alcohol denaturation disrupts the hydrogen bonds of the globular protein structure, whilst instead forming new hydrogen bonds between its polypeptide chains (Brandts and Hunt, 1967, Nikolaidis and Moschakis, 2018, van Koningsveld et al., 2002). In terms of the mechanism of interaction with aroma compounds, this corresponds directly to a decrease in hydrophobic pockets, which correlates with the shift in the intensity to more hydrophobic aroma compounds in the 5% ABV beers. These changes in the hydrodynamic properties of salivary proteins, including higher viscosity and changes in conformation are suggested to be strongly correlated with the changes in the sensorial perception of beer, including flavour and mouthfeel effects confirmed through the in-mouth flavour evaluation. Similar changes in the hydrodynamic properties of salivary proteins are suggested to be responsible for a specific flavour profile i.e. more fruity/estery hydrophobic compounds such as linalool, ethyl hexanoate and isoamyl acetate in the 5% ABV. Conversely, more worty/malty compounds such as the more hydrophilic furfural, ethyl acetate and 3-methylbutanal appeared to be more enhanced in the absence of ethanol, in both beer styles.

Together, these findings illustrate the importance of linking sensory data with analytical techniques in order to enhance the current understanding of physicochemical changes occurring during food and beverage oral processing, also highlighted in Ickes and Cadwallader (2017). In particular, the combined

approach is instrumental for the analysis of intra-oral interactions, which offers brewers a new opportunity for matrix design with controlled oral processing characteristics, flavour release and perception of beer. For NABs, the understanding of the dynamics of flavour release is particularly important for replacing the lost functionality of ethanol and unlocking new dimensions in formulation design. It was suggested that some of the lost functionality of ethanol may be tackled by the addition of dextrins or glycerol which can act as 'ethanol-mimics' and help increase aldehyde retention (Perpete and Collin, 2000). Further research into oral mucoadhesives might become an attractive option in beer reformulation, by modulating an increase in the retention of more hydrophobic compounds (Dinu et al., 2019a). As observed in Dinu et al. (2019a) the development of oral mucoadhesives can lead to a decrease in the interactions of aroma compounds with α-amylase. Balancing these effects could provide brewers with significant guidance on the development of a NAB base recipe, in order to reduce the effects of beer dealcoholisation.

3.3.1 Concluding remarks and future work

In an attempt to provide an integrated approach in evaluating perceptual and physical changes during consumption of 0% ABV beverages, this study used consumer sensory evaluation, GC-MS analysis and hydrodynamic protein analysis. The aim was to understand the impact of ethanol (0 and 5% ABV), saliva and their interactions on the perception of two different beer styles: lager and stout. Firstly, consumer sensory evaluation demonstrated that orthonasal perception of aroma alone is not enough to allow significant discrimination between the 0% and 5% lagers. However, during in-mouth assessments, discrimination of flavour, taste and mouthfeel attributes in 0% and 5% beer was

possible, as evidenced from the TCATA analysis. This suggested an ethanol*saliva interaction effect and provided evidence that this complex interaction can affect the sensory attributes of lager. The phenomenon appeared to influence the flavour profile of 0% ABV beer, which shifted to more hydrophilic molecules, while the 5% ABV samples had a higher relative proportion of more hydrophobic compounds. This effect was observed in both lager and stout beer types and was linked to ethanol denaturation of salivary proteins, resulting in an extended polypeptide which has fewer hydrophobic pockets that can trap aroma molecules. Further mechanistic investigations are suggested, particularly using other key components in our saliva such as mucins, PRP's and other glycoforms of α-amylase.

3.4 Materials and Methods

3.4.1 Consumer Sensory Analysis

3.4.1.1 Participants

To assess the influence of ethanol on perception of beer, 101 consumers (53 men, 48 women), who self-reported consumption of beer at least once every two months, were recruited to take part. Ages ranged from 19 to 70 years of age, with a mean age of 32. Approval from the University of Nottingham Medical Ethics Committee (G10022017) was granted before the study commenced and research was performed in accordance with the Institute of Food Science and Technology Guidelines for Ethical and Professional Practices for the Sensory Analysis of Foods. All participants gave written informed consent to participate in the study and were offered an inconvenience allowance for their time.

3.4.1.2 Samples

A 0% ABV lager (Carlsberg, Northampton, UK) was used as base beer from which two experimental beer samples (0 and 5% ethanol) were prepared. To create the 5% ethanol beer samples, 30 mL of ethanol water mixture (18.09 mL of 96% food grade ethanol (VWR International, Lutterworth, UK) and 11.91 mL of water (Danone, Paris, France) was added to 300 mL of commercial beer. To create the 0% ethanol beer samples, 30 mL of water was added to ensure that all samples had the same concentration of matrix components. On the day of testing, 30 mL of beer was removed from a 330 mL commercial bottle, and the desired ethanol/water solution was added back, with inversion of the bottle to ensure adequate mixing. A lager style beer was chosen for this part of the study, as this is the beer style with the largest market and so there is a larger commercial relevance. For evaluation by consumers, 30 mL of beer was poured into plastic serving cups and served, with each bottle prepared serving no more than 10 consumers. This method was used to minimise sample handling and limit the decarbonation and volatilisation of the samples.

3.4.1.3 Procedure

Consumers participated in the study at the Sensory Science Centre, Sutton Bonington Campus, University of Nottingham, with tests performed at room temperature in an air-conditioned room, under Northern Hemisphere daylight and in individual booths, which conform to ISO standards (ISO 8589: 2007). Data was collected using Compusense software (Guelph, Ontario, Canada).

The session started in a discussion room, where a familiarisation task (15 min) took place. Previous research has shown that familiarising consumers with

the methods used to assess products can result in an increase in the ability of consumers to discriminate amongst samples (Jaeger et al., 2017). Consumers were also familiarised with the attributes and definitions they would be using (shown in Appendix Table 2). Further details on attribute generation are discussed in section 3.4.1.4. Consumers then evaluated samples in isolated sensory booths (45 min). Check-All-That-Apply (CATA) was used to assess orthonasal aroma attributes and Temporal Check-All-That-Apply (TCATA) was used for in-mouth assessments, including taste, flavour, mouthfeel and aftertaste.

Beer samples (n=2) were presented monadically under Northern hemisphere lighting, in a randomised order, according to a Williams Latin Square Design (Meyners et al., 2013). The attribute order was also randomised across subjects to balance bias associated with list order for both CATA and TCATA attributes. The attribute list order was consistent for a given panellist across all samples (Meyners and Castura, 2016). Data were captured using Compusense© Cloud software (Guelph, Ontario, Canada). To minimise fatigue and carryover, consumers were given a forced 2 min break between each sample, and were told to take at least 2 sips of water during this break to cleanse the palate.

3.4.1.4 Check-all-that-apply (CATA) – Orthonasal Pathway

Consumers were asked to assess the presence of six aroma attributes within each sample with the use of a predefined CATA checklist. The attribute list and definitions were generated after a pilot study with six naïve beer consumers (see Appendix Table 2). Consumers were advised to take 2-3 short sharp sniffs of the sample and then a longer sniff before clicking on the attributes they perceived.

3.4.1.5 Temporal Check-All-That-Apply (TCATA) – In-Mouth Flavour Pathway

Consumers were then asked to assess the presence of 10 predefined attributes within each sample using TCATA, which is a developed sensory method focusing on all attributes, not just dominant, in the sample over time. This method was chosen for in-mouth assessments such as flavour, taste and mouthfeel as beer has a complex profile which changes over consumption time. Ten attributes were selected so as not to exceed the recommended maximum for consumers (Pineau et al., 2012). Attributes and definitions were developed in reference to published literature (Langstaff and Lewis, 1993, Martin and Pangborn, 1970, McMahon et al., 2017, Meilgaard et al., 1979). Prior to the test, consumers were instructed to familiarise themselves with the position of the attributes on screen, which were presented in a three-column format.

3.4.2 Physicochemical Analysis

3.4.2.1 Samples

A 0% ABV lager (Carlsberg, Northampton, UK) and a 0% ABV stout (Big Drop Brewing Co, Ipswich, UK) style beer were used as base beers from which two experimental beer samples (0 and 5% ethanol) were prepared, as in section 3.4.1.2. These samples were then spiked with a flavour cocktail for GC-MS measurements in order to achieve adequate signal. The volatile compounds used included: aldehydes (3-methyl butanal, furfural and hexanal), esters (ethyl acetate, ethyl hexanoate and isoamyl acetate) and alcohols (isoamyl alcohol, linalool and phenylethyl alcohol) (Sigma Aldrich, Dorset, UK) selected due to their contribution to beer flavour, as well as differences in chemical properties. A stock solution of these compounds was made in 95% ethanol and this was then

transferred into the ethanol/water mixtures to ensure consistency. Added concentrations were as follows: ethyl acetate (8.44mg/L), isoamyl acetate (0.40mg/L), ethyl hexanoate (0.41mg/L), isoamyl alcohol (40.78mg/L) phenylethyl alcohol (9.96mg/L), hexanal (0.81mg/L), furfural (5.99mg/L), 3-methyl butanal (4.07mg/L) and linalool (0.92mg/L). These concentrations are typically found in lager beer for these compounds (Briggs et al., 2004). Physicochemical characteristics for all of these compounds can be found in Table 3.2. Samples were stored at 4 ± 2 °C prior to sampling.

Table 3. 2: *Hydrophobicity of flavour compounds (LogP) and their sensory descriptors (Flavournet, 2004).*

Volatile Compound	***Log P***	***Flavour in Beer***
Furfural	0.83	Bread, almond, sweet
Ethyl Acetate	0.86	Solvent, fruity, pineapple
3-Methyl Butanal	1.23	Malt
Isoamyl Alcohol	1.26	Whiskey, malt, burnt
Phenylethyl Alcohol	1.57	Honey, spice, rose, lilac
Hexanal	1.80	Grass, tallow, fat
Isoamyl Acetate	2.26	Banana, apple, solvent
Ethyl Hexanoate	2.83	Apple peel, fruit
Linalool	3.38	Flower, lavender

3.4.2.2 α-Amylase solution preparation

The α-amylase solution was made by preparing 10 mg/mL α-amylase from *Bacillus licheniformis* (Sigma A4551) in 0.1 M phosphate buffered saline

(Sigma Aldrich, Dorset, UK) (Green, 1933). The concentration of buffer and amylase were chosen to mimic the concentration of salivary α-amylase and electrolytes in saliva (Mandel et al., 2010).

3.4.2.3 Gas Chromatography Analysis

To detect volatile compounds, Solid Phase Microextraction Gas Chromatography Mass Spectrometry (SPME-GC-MS) was used. Beer samples (2 mL) and either buffer or α-amylase solution (2 mL) were transferred into glass vials at a 1:1 ratio. The vials were left to equilibrate for 3 hours before analysis. Analysis of volatile aroma compounds was performed using a Trace 1300 series Gas Chromatograph coupled with a single-quadrupole mass spectrometer (Thermo Fisher Scientific, Hemel Hempstead, UK). The method used was modified from Yang et al. (2016). Briefly, samples were incubated at 40°C for 2 min with shaking. A 50/30 μm multiphase SPME Fibre (PDMS/DVB, Supelco, Sigma Aldrich, UK) was used to extract volatile aroma compounds from the sample headspace (extraction for 10 min then desorption for 1 min). The injector temperature was set at 200 °C in splitless mode (constant carrier pressure 18 psi (124 kPa). Separation was carried out on a ZB-Wax capillary GC column (30m x 0.25 ID; Phenomenex Inc, Cheshire, UK). Column temperature was held initially at 40 °C for 2 min, increased by 8 °C/min to reach 240 °C and held for 1 min. Full scan mode was used to detect volatile compounds (mass range from m/z 35 to 200). Volatile compounds were identified by comparison of each mass spectrum with either the spectra from authentic compounds analysed in the laboratory or with spectra in reference collections (NIST Mass Spectral laboratory).

3.4.2.4 Sedimentation Velocity- Analytical Ultracentrifugation

The effect of ethanol on sedimentation velocity of α-amylase was examined using the Optima XL-I analytical ultracentrifuge (Beckman, Palo Alto, USA) equipped with Rayleigh interference optics. For the sedimentation experiments, 395 µL and 405 µL aliquots of solution and solvent, respectively, were injected into the 12mm double sector epoxy cells with sapphire windows and run at 40000 rpm (120 000 g) at 20 °C. The results were analysed in SEDFIT using the c(s) processing methods by generating sedimentation coefficient distributions, $s_{20,w}$ (in Svedberg units, S = 10^{-13} sec) normalised to standard conditions (viscosity and density of each solvent at 20°C).

3.4.2.5 Ostwald capillary viscometer

Flow times of the respective ethanol/water solvents (t_0) and α-amylase solutions (t_s) were measured using the semi-automated (Schott Geräte, Hofheim, Germany) U-tube Ostwald capillary viscometer immersed in a temperature controlled water bath at 20°C. A constant volume of 2 mL was sampled at constant α-amylase concentration of 10 mg/mL. The intrinsic viscosity, [η] was calculated according to the Solomon-Ciuta equation (3.1) (Solomon and Ciută, 1962):

$$[\eta] \cong \frac{1}{c}\left(2\left(\eta_{sp}\right) - 2ln(\eta_r)\right)^{1/2} \qquad (3.1)$$

3.4.3 Data Analysis

3.4.3.1 Consumer Data: CATA and TCATA

3.4.3.1.1 CATA

Analysis of CATA data followed previous work by Meyners et al. (2013). This was performed by counting the number of assessors that checked

each given attribute, forming a contingency table. Cochran's Q analysis with Bonferroni as a multiple comparison was then performed to show significant differences among samples for each aroma term.

3.4.3.1.2 TCATA

The analysis of the average proportion of citations followed a similar method as McMahon et al. (2017), with each attribute being assessed as the proportion of the 60 s time period in which it was selected. For example, if malty was checked for a duration of 15 s and hoppy for 25 s, the proportion of citations for malty would be 15/60 = 0.25 and for hoppy would be 25/60 = 0.42. A two factor ANOVA (sample, panellist) and Tukey's HSD post hoc test was then performed to understand the significance of each attribute.

3.4.3.2 GC-MS

To calculate the separate effect of ethanol and α-amylase interactions with beer, all GC-MS samples were analysed in 4 replicates, using a one-way analysis of variance (ANOVA) and Tukey's post hoc test to identify significance ($p < 0.05$). The percentage changes were then calculated, relative to their controls. For instance, for the effect of ethanol, the 0% samples were considered controls and for the effect of saliva, the water samples were controls. To quantify the effect of α-amylase interactions with different ethanol beers, a two-way ANOVA with Tukey's post hoc test was performed to understand the interactions of ethanol and saliva on the two different beer styles, with Pearson's correlation coefficient calculated to construct a correlation map to understand the relationship between factors.

3.4.3.3 Hydrodynamics

The theory of Scheraga and Mandelkern (Scheraga and Mandelkern, 1953) was applied to evaluate molar mass using experimentally determined sedimentation coefficient distribution and intrinsic viscosity. The model assumes that a macromolecule can be represented by an ellipsoidal shape, using the following equation (3.2):

$$M = \left(\frac{N_A s^{\circ}_{20,w} [\eta]^{1/3} \eta_o}{\beta(1-\bar{\nu}\rho_o)100^{1/3}}\right)^{3/2} \qquad (3.2)$$

Where M is molar mass (g/mol), N_A is Avogadro's constant (mol^{-1}), $[\eta]$ is the intrinsic viscosity, η_o is solvent viscosity, $s^{\circ}_{20,w}$ is sedimentation coefficient distribution, $\bar{\nu}$ is the partial specific volume of the protein, ρ_o is the density of the solvent (g/cm^3) and β is a shape function, ranging from $2.11 \cdot 10^6$ for spheres to $2.55 \cdot 10^6$ for elongated molecules. As the molecular weight of α-amylase is known, the formula was rearranged in order to obtain the shape function β, which is used for the determination of the axial ratio of a prolate ellipsoid in the program ELLIPS 1 (García de la Torre and Harding, 2013).

4 Understanding the sensory and physicochemical differences between commercially produced non-alcoholic lagers, and their influence on consumer liking

Preliminary thoughts Chapter 4:

Chapters 2 and 3 explored the impact of the simple addition of ethanol into a NAB matrix, to offer an increased understanding into the impact of ethanol on flavour release and perception. Results in Chapter 3 discovered that decreased ethanol concentration increased headspace intensity of volatile compounds through GC-MS analysis, however this effect was not shown to effect orthonasal sensory evaluation results and instead only in-mouth assessments interaction. The presence of salivary proteins at lower ethanol concentrations appeared to replicate the changes in-mouth during consumption, causing a shift to more hydrophilic compounds. These included compounds such as 3-methyl-butanal and furfural, known to be present in high concentrations in NAB. In-mouth sensory evaluation results confirmed these findings, with the 0% beer perceived as maltier. Aldehydes have previously been found to be responsible for this worty/malty flavour in NAB due to

decreased retention of these compounds in the absence of ethanol. The 5% beer was also perceived as fruitier, which is linked to the increased presence of more hydrophobic esters such as ethyl acetate and ethyl hexanoate. In addition, the 5% beer was found to have increased sweetness, fullness/body and alcohol warming sensation. Chapter 2 discovered that these attributes can impact consumer liking, with clustering of consumers revealing that this was either positive or negative dependent on the consumer. The largest cluster of consumers (C2), were found to enjoy the attributes of malty, alcohol warming sensation and sweet, therefore showing that these are all important sensory characteristics driving consumer liking in NABs. It would therefore be interesting to explore consumer liking of commercial NABs and their sensory and physicochemical characteristics.

As discussed in chapter one (section 1.3), there are numerous techniques used to produce NAB, ranging from biological to physical methods, as well as a combination of the two. Some studies have suggested that the resulting sensory and physicochemical properties of NAB can be characterised by production method. Indeed, many of the studies reviewed in chapter one (section 1.3) reported changes between the original and NAB in terms of basic brewing parameters (such as colour, bitterness units, density) as a result of production method. However, only a handful reported chemical changes using GC-MS and even less conducted robust sensory analysis, resulting in a need for a study exploring the sensory properties, consumer liking and physical properties of commercial beers made using a wide range of production methods.

The following study aimed to understand the differences between commercial NABs using physicochemical and sensorial techniques, and the effect these have on consumer liking. Resulting data was discussed in relation to production method to understand whether certain methods yield different sensory and physicochemical profiles that impact consumer liking.

This chapter was submitted as a paper to Food Chemistry X in June 2020 and accepted for publication in January 2021:

Ramsey, I., Yang, Q., Fisk, I., and Ford. R. (2020) Understanding the sensory and physicochemical differences between commercially produced non-alcoholic lagers, and their influence on consumer liking. *Food Chemistry X*, 9, 100114.

doi: https://doi.org/10.1016/j.fochx.2021.100114

Understanding the sensory and physicochemical differences between commercially produced non-alcoholic lagers, and their influence on consumer liking

Imogen RAMSEY[ab], Qian YANG[a], Ian FISK[b] and Rebecca FORD[a]*

[a]Sensory Science Centre, Division of Food, Nutrition and Dietetics, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, United Kingdom

[b]Samworth Flavour Laboratory, Division of Food, Nutrition and Dietetics, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, United Kingdom

Key words: Non-Alcoholic Beer, Production Methods, QDA, Consumers

Highlights:

- Variation in sensory and physicochemical profiles not explained by production method.
- Differences instead were proposed to be due to pre and post processing methods.
- Overall consumer liking could be optimised by mixing different production techniques.
- Five patterns of consumer liking identified, related to sensory characteristics.

Abstract:

This study aimed to investigate the sensory and physicochemical differences of a range of commercial non-alcoholic lagers, as well as their influence on overall liking. Using physicochemical analysis and modified quantitative descriptive analysis (QDA) with a trained panel (n=10) eighteen commercial non-alcoholic lagers, made using different production methods, were assessed. A subset (eleven), representing the sensory space were also assessed for hedonic liking using consumers (n=104). Overall, it showed a clear variety of non-alcoholic lagers were selected, with different clusters of samples found with identifiable characteristics. Production methods were explored as a possible explanation for the differences in characteristics, however these did not fully explain the clusters and therefore other factors, such as pre or post processing methods are discussed. In terms of overall liking, five clusters of consumers were discovered with different patterns of liking, confirming that a wide range of non-alcoholic lagers are needed to satisfy all consumers.

4.1 Introduction

The international non-alcoholic beer (NAB) market is predicted to experience a rise in total volume growth of 24% by 2021 and be worth over $25bil by 2024 (Verma and Rawat, 2018), showing its value and importance in the drinks sector (Euromonitor, 2017b). Interest in these products in the Middle East, Africa and Western Europe appear to be the drivers of this growth, with countries such as Germany owning 14% of the worldwide non-alcoholic drinks market (Euromonitor, 2017b).

This increase in value is down to many factors, with 47% of consumers limiting their alcohol consumption compared to 12 months earlier (Mintel, 2019b) and an increased drive from global manufacturers to emphasise responsible drinking (ABInBev, 2018). These factors have led to the consumer moderating their alcohol consumption focusing on improving health, weight management and saving money (Mintel, 2017a). The biggest challenge for breweries is to produce lower alcohol variants which taste more like their standard strength equivalents, with one in three consumers claiming this would sway them to drink more of these products (Mintel, 2017a). Therefore, an opportunity has arisen for the growth of the low and non-alcoholic drinks sector, leading to an increase in the development of lower alcohol alternatives. One of the most interesting developments in this ever changing field is the introduction of craft breweries solely focusing on the production of low alcohol/NAB (Euromonitor, 2017b), resulting in increased experimentation, innovation and development. Much of this innovation focuses on different production methods to produce appealing sensory profiles (Euromonitor, 2017b).

The production of NABs can be divided into two main categories: biological and physical methods. Biological methods focus on limiting ethanol production early on in the process, whilst physical methods remove ethanol post brewing. Different techniques are summarised in Figure 4.1, with comprehensive reviews provided by Branyik et al. (2012) and Bellut and Arendt (2019). Biological methods can be split into those that use traditional brewing equipment (arrested or limited fermentation, altered mashing and special yeasts) and those that need specialist equipment (continuous fermentation). Previous studies have suggested that these techniques can cause decreases of up to 87% for esters and 80% for higher alcohols in comparison to original beers (Narziß et al., 1992), resulting in a disharmonious final beer product, with wort-like off flavours and increased sweetness (Sohrabvandi et al., 2010). However, there has been limited sensorial research characterising these properties. Detailed reviews on the physical methods of creating NABs, including industrial scale thermal based processes, such as spinning cone column (SSC) and vacuum distillation, have shown acceptable final products with reduced thermal stress (Branyik et al., 2012, Müller et al., 2017, Zufall and Wackerbauer, 2000b). However, studies comparing the losses of volatiles by these methods found up to 100% of esters and up to 98% higher alcohols were lost in comparison to the original beer (Branyik et al., 2012, Zufall and Wackerbauer, 2000b). Membrane processes include; dialysis, reverse osmosis (RO), osmotic distillation (OD), nanofiltration (NF) and pervaporation.

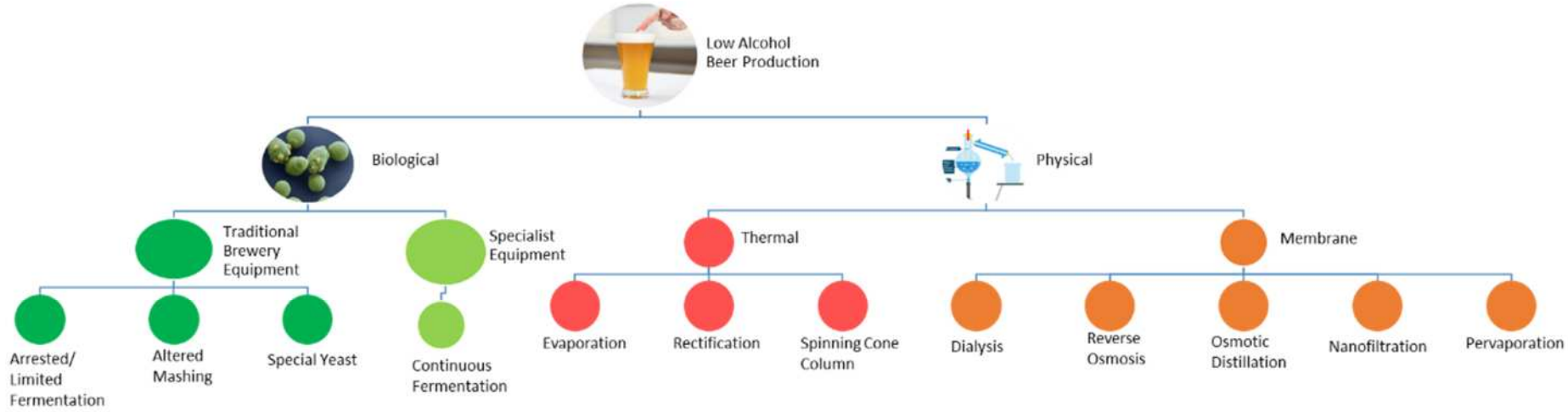


Figure 4.1: *Non-alcoholic beer production methods. Green indicates biological methods, including traditional brewery equipment and specialist equipment. Physical methods are also shown, with red indicating thermal based methods and orange indicating membrane based technologies*

To the authors knowledge, only two of these processes (dialysis and RO) are used on an industrial scale (Branyik et al., 2012) yet still result in large reductions in esters and higher alcohols (up to 87% and 81% respectively) (Kavanagh et al., 1991, Stein, 1993). The sensory properties of NABs made by both thermal and membrane based processes have resulted in beers described as having less aroma and body and more acidity (Montanari et al., 2009). To counteract this, some breweries have attempted to combine both biological and physical methods to produce a more sensorially acceptable NAB (Jiang et al., 2017).

The production method chosen to produce a NAB has previously been shown to impact the sensory qualities of beer (Krebs et al., 2018, Schmelzle et al., 2013). Research by Schmelzle et al. (2013) used descriptive analysis with semi-trained consumers to describe sensory differences amongst twelve samples produced through different techniques and they were able to divide them into 'physical 'and 'biological and mixed methods'. In another study, the impact of production technique on the macromolecular profile of commercial NABs was studied (Krebs et al., 2018) but only mouthfeel sensory descriptors and physical instrumental information was provided. Due to technological advances and the combining of methods, further research is required to investigate the sensory and physicochemical impact of a wide range of techniques that are currently being used within the brewing industry. Whilst several studies have investigated the loss of volatile compounds using different production techniques (Bellut and Arendt, 2019, Müller et al., 2017), only one has looked at the effect on the sensory properties of beer, as well as on consumer liking (Schmelzle et al., 2013), which is critical for the brewing

industry. The relationship between sensory characterisation and flavour chemistry would further advance knowledge regarding production of NAB, therefore guiding breweries towards practices they can use to improve the quality and consumer liking of their products.

The objectives of this study were therefore to investigate the physicochemical and sensorial properties of a range of commercially produced non-alcoholic lager style beers. This was achieved through developing a robust category wide non-alcoholic lager sensory lexicon using a trained sensory panel, whilst also correlating sensory data with physicochemical properties to reveal relationships for the wider category. Beers were clustered to understand sensorial similarities and differences, and possible effects of production method were explored to ascertain whether they had an effect on the overall characteristics of the beer, or whether other parameters were the source of these differences. Finally, the influence of these sensory properties on consumer liking were assessed.

4.2 Materials and Methods

4.2.1 Samples

A range of non-alcoholic commercial lagers (n=18) from the EU market were carefully selected to include a wide range of flavour characteristics and production methods (discovered by either intellectual property or from brand websites). The production methods were split into five categories, which included: altered brewing, special yeasts, dealcoholized (samples that used thermal or membrane based technologies), vacuum distillation and mixed

methods (samples that underwent both biological and physical processing). Details are shown in Table 4.1. Samples were kept in cold storage at 4±2 °C before assessments commenced.

4.2.2 Physicochemical Analysis

Instrumental analyses were conducted to investigate the differences in the commercial non-alcoholic lager style beers and their key chemical characteristics. Ethanol content was measured using an Anton Paar Alcolyzer and DMA4500 (Graz, Austria). Sample pH was determined using a Metler Toledo FiveGo pH meter (Colombus, Ohio, USA) after calibration with pH 4.0 and 7.0 standards. Bitterness units (BU) were determined using the international method proposed by the American Society of Brewing Chemists (ASBC) (Beer-23A) (ASBC Method of Analysis, 2018). Beer (5 mL) was transferred into a 50 mL centrifuge tube and acidified with 3 M HCl (0.5 mL). Isooctane (10 mL) was added and the mixture was shaken by hand three times and then placed on a mechanical shaker for 15 min. The mixture was subsequently centrifuged at 400 x g for 5 min, and then again for another 5 min to aid phase separation. The clear isooctane layer was then transferred into a cuvette and absorbance was measured at 275 nm with a spectrophotometer against a blank of isooctane. The recorded absorbance was multiplied by 50 to give BU values in mg/L. Total polyphenol (TP) content was also determined using the international method proposed by the ASBC (Beer-35) (ASBC Method of Analysis, 2015). Beer (10 mL) was mixed with a preparation of carboxymethylcellulose (CMC, 1%) and ethylenediamine tetraacetic acid (EDTA, 0.2%) (8 mL) in a 25 mL volumetric flask. Ferric acid (0.5 mL) and ammonia (0.5 mL) were then added, with mixing after each addition.

Table 4.1: *Beer samples, production methods, size of brewery, additional ingredients and physicochemical analysis results. Size of brewery is described as either M (multinational brewery) or C (craft brewery). Additional ingredients were those described on commercial beer labels, which included anything other than water, barley malt, yeast and hops. Different letters within a column[abc] represent a significant difference among samples in terms of physicochemical parameters (Tukeys HSD, $p<0.05$). Samples with an asterisk (*) were those selected for the subset for consumer overall liking sensory analysis.*

Sample Number	Production Method	Size of Brewery	Additional Ingredients	Ethanol Content (ABV)	pH	Bitterness Units (BU)	Total Polyphenols (mg/L)	Fermentable Sugars (g/L)				
								Glucose	Sucrose	Fructose	Maltose	Maltotriose
1*	Altered Brewing	M	Wheat	0.05^{efg}	4.48^{c}	18.29^{b}	49.20^{h}	2.79^{abc}	0.20^{c}	0.86^{cde}	12.64^{a}	5.13^{a}
2*	Altered Brewing	C	Rye, Wheat, Maltodextrin	0.57^{b}	4.81^{a}	17.38^{bc}	114.80^{cd}	0.04^{f}	0.01^{c}	0.02^{g}	0.00^{e}	1.41^{cde}
3*	Altered Brewing	M	Corn	0.03^{g}	4.44^{cde}	13.68^{defg}	114.16^{cd}	1.69^{cde}	0.19^{c}	0.91^{cd}	9.35^{abcd}	3.39^{abc}
4	Altered Brewing	M	Flavouring	0.12^{e}	4.14^{gh}	15.44^{cd}	115.71^{cd}	3.04^{ab}	0.30^{bc}	1.17^{c}	10.17^{abc}	3.71^{abc}
5*	Special Yeast	M	Modified hop products	0.06^{efg}	4.41^{de}	12.49^{fg}	119.90^{c}	3.18^{a}	1.11^{a}	0.92^{cd}	12.77^{a}	4.59^{ab}
6*	Special Yeast	M	N/A	0.49^{b}	4.10^{h}	13.59^{defg}	79.18^{fg}	0.21^{f}	0.06^{c}	0.05^{fg}	9.80^{abc}	3.95^{abc}

7*	Dealcoholised	M	N/A	0.05^{efg}	4.31^{f}	25.34^{a}	118.26^{c}	0.09^{f}	0.18^{c}	0.09^{fg}	3.85^{cde}	2.22^{abcde}
8	Dealcoholised	M	Hop extract	0.08^{efg}	4.46^{cd}	18.59^{b}	153.61^{b}	0.06^{f}	0.18^{c}	0.01^{g}	0.00^{e}	0.13^{e}
9	Dealcoholised	M	Rice, Malt extract, Hop extract, Natural flavours	0.07^{efg}	4.40^{de}	5.26^{i}	91.11^{ef}	0.63^{ef}	0.14^{c}	0.24^{fg}	2.90^{de}	1.20^{cde}
10	Dealcoholised	M	Sugar, Natural flavourings	0.08^{efg}	4.13^{gh}	11.34^{gh}	71.25^{g}	2.88^{abc}	0.29^{bc}	3.12^{a}	0.00^{e}	0.00^{e}
11	Vacuum Distillation	M	Maize, Rice	0.03^{fg}	4.10^{h}	13.62^{defg}	112.89^{cd}	2.91^{abc}	0.34^{bc}	1.06^{c}	4.61^{bcde}	2.61^{abcde}
12*	Vacuum Distillation	C	N/A	0.75^{a}	4.27^{f}	14.21^{def}	235.98^{a}	0.07^{f}	0.23^{c}	0.08^{fg}	1.32^{e}	1.65^{bcde}
13*	Vacuum Distillation	C	N/A	0.35^{cd}	4.19^{g}	17.94^{b}	98.22^{def}	0.04^{f}	0.07^{c}	0.02^{g}	0.21^{e}	0.38^{de}
14	Vacuum Distillation	M	Unmalted barley, Corn, Flavouring	0.39^{c}	4.15^{gh}	9.77^{h}	154.62^{b}	0.11^{f}	0.19^{c}	0.04^{fg}	0.65^{e}	1.36^{cde}
15*	Mixed Methods	C	N/A	0.31^{d}	4.38^{e}	15.36^{cde}	161.90^{b}	2.18^{abc}	0.25^{c}	0.94^{cd}	8.76^{abcd}	3.28^{abcd}
16*	Mixed Methods	M	Hop extract, Natural flavourings	0.12^{e}	4.46^{cd}	13.74^{defg}	152.70^{b}	0.90^{def}	0.23^{c}	0.41^{efg}	3.78^{cde}	1.41^{cde}
17*	Mixed Methods	M	Corn	0.03^{g}	3.99^{i}	13.95^{def}	109.88^{cde}	2.05^{abcd}	0.11^{c}	2.00^{b}	0.18^{e}	0.17^{e}
18	Mixed Methods	M	Maize, Natural flavourings	0.11^{ef}	4.68^{b}	12.94^{efg}	92.30^{ef}	1.90^{bcde}	0.70^{ab}	0.50^{def}	10.69^{ab}	3.44^{abc}

The solution was then made up to the mark with RO water, left to stand at room temperature for 10 min, and absorbance was measured at 600 nm with a spectrophotometer against a blank of the beer sample (mixed with CMC/EDTA and ammonia). The recorded absorbance was multiplied by 820 to give total polyphenol values in mg/L. Fermentable sugars were determined via high-performance liquid chromatography (HPLC) using Dionex ICS-3000 Reagent-Free Ion Chromatography, electrochemical detection using ED40 and computer controller. The CarboPac PA20 column (3x150mm) was used, and the mobile phase was 10 mM NaOH with a flow rate of 0.5 mL/min. The injection volume was 10 µL and the column temperature was 30 °C. This method was modified from Kostas et al. (2016). Authentic standards of sugars (maltose, sucrose, fructose, maltotriose, glucose) (Sigma-Aldrich Ltd, Dorset, UK) were used for quantification.

Headspace Gas Chromatography Flame Ionization Detector (HS-GC-FID) lower boiling point beer volatile analysis was determined using the method proposed by Analytica-European Brewing Convention (EBC) (9.39) (Analytica-EBC, 2018). Beer samples (10 mL) were transferred into glass vials with 3.5 g sodium chloride and 50 µL 1-butanol (internal standard). Volatiles were analysed with a Scion 456-Gas Chromatograph (Scion Instruments, West Lothian, UK). Samples (500 µL) were incubated at 60 °C for 20 min with shaking, and then were injected in splitless mode using a PAL Combi-XT autosampler (PAL System, Zwingen, Switzerland) onto a Zebron ZBWax column (60m x 0.25 ID; Phenomenex Inc, Cheshire, UK). Column temperature was held initially at 85 ˚C for 10 min, increased by 25 ˚C/min to 110 ˚C, before finally being increased by 8 ˚C/min to 200 ˚C. Total run time was 36.25 min.

The GC carrier gas was helium, at a constant pressure of 15 psi. Full scan mode was used to detect volatile compounds (mass range from m/z 35 to 200). Volatile compounds were identified by their m/z, and quantified with the use of pure and internal standards. The following aroma compounds were purchased from Sigma-Aldrich (UK) for standard identification: acetaldehyde (≥99.5%), ethyl acetate (≥99.5%), isobutyl acetate (2-methylpropyl ethanonate) (≥97%), propan-1-ol (≥99%), isoamyl acetate (3-methylbutyl acetate) (≥97%), 3-methyl-1-butanol (≥99%), ethyl octanoate (≥98%) and ethyl decanoate (≥98%). Other compounds were purchased from Thermo Fisher Scientific (UK): 1-butanol (≥99.5%), ethyl butanoate (≥99%), 2-methylpropan-1-ol (≥99%) and ethyl hexanoate (≥99%).

To detect other relevant volatile compounds not found through HS-GC-FID analysis, Solid Phase Microextraction (SPME) was used. Beer samples (5 mL) were transferred into glass vials and 100 µL 3-heptanone (internal standard) was added and analysed using a modified published method by Yang et al. (2016). Modifications to the method included incubation of samples at 40 °C for 2 min with shaking, with volatile aroma compounds extracted for 10 min and desorped for 1 min. Column temperature was held initially at 40 °C for 2 min, increased by 8 °C/min to 240 °C and held for 1 min. Total run time was 38 min. Full scan mode was used to detect volatile compounds (mass range from m/z 35 to 200). Volatiles were identified by their m/z and comparison of each mass spectrum with either the spectra from authentic compounds or with spectra in reference libraries (NIST/EPA/NIH Mass Spectral Library, version 2.0, Faircom Corporation, U.S.) The quantification of volatiles collected from the headspace was expressed by the peak area ratio

(PAR), which was calculated by the GC peak area for the compound divided by the peak area of the internal standard.

4.2.3 Sensory Analysis

Approvals from the University of Nottingham Medical Ethics Committee for both Quantitative Descriptive Analysis (QDA) and the Consumer Study (approval codes: 163-1812 and 328-1906) were granted. All participants gave written informed consent to participate and were offered an inconvenience allowance for their time. All tests took place at the Sensory Science Centre, Sutton Bonington Campus, University of Nottingham in individual booths conforming to ISO standards (ISO 8589: 2007). Data was collected using Compusense software (Guelph, Ontario, Canada).

4.2.3.1 Quantitative Descriptive Analysis

The sensory attributes of eighteen commercial non-alcoholic lager style beer samples were evaluated by trained beer panellists (n=10, 4 male, 6 female) using a modified QDA approach. Panellists were trained over twenty one, two-hour sessions. Initial training sessions identified and evaluated aroma, taste, flavour and mouthfeel attributes for all commercial beer samples using attribute generation. Subsequent training sessions expanded the attribute list, with definitions and reference standards for each attribute (data not shown here, see Appendix Table 3). Only attributes which the panel agreed on by consensus and that discriminated amongst samples were used. These attributes and definitions were developed in reference to published literature (Langstaff and Lewis, 1993, Meilgaard et al., 1979). All attributes were evaluated using a continuous unstructured line scale, with marks converted to a score of ten for data analysis purposes. Panellist performance was continually monitored for

discrimination, consistency and repeatability using blind replicate samples and samples spiked with reference standards. Retraining was conducted where necessary. Final sample evaluation started once the panel demonstrated adequate repeatability and discrimination.

Samples were evaluated in nine 2 hr sessions over two months, allowing for triplicate evaluations of each sample by each panellist. Beer samples, labelled with three-digit codes, were served at 4±2°C and presented in a balanced, blocked and randomised presentation order, with 2 min breaks between each sample. Panellists were provided with three bottles of each sample (3 x 20 mL) to ensure temperature was kept constant throughout assessment and beers were fresh. Panellists were instructed to use their first bottle for aroma, with subsequent bottles being used for flavour, taste and mouthfeel attributes. The order of attributes was agreed with panellists before final evaluation took place, starting with the attribute that was perceived first and ending with the last. A maximum of seven samples were evaluated per two-hour session to ensure no carryover or fatigue effects. Unsalted crackers (Rakusens, Leeds, UK), honeydew melon (Sainsburys, Milton Keynes, UK) and Evian mineral water (Danone, Paris, France) were provided for palate cleansing.

4.2.3.2 Consumer Liking Analysis

Consumers (n=104, 47 men, 57 women), who self-reported consumption of beer at least once a month participated in the study. A subset of the samples (n=11) were selected after analysis of the QDA data to represent samples with a wide range of sensory characteristics produced by different production methods (shown in Table 4.1). All consumers participated in two

evaluation sessions over two weeks. Both sessions collected overall liking (OL) data using a 9-pt hedonic scale ranging from ‘dislike extremely’ to ‘like extremely’ with consumers rating six samples per session. In each session, samples were presented monadically using a randomised balanced design according to a Williams Latin Square. To minimise fatigue and carryover, consumers were given a forced 1 min break between each sample, and were told to take at least 2 sips of water (Evian, Danone, France) and consume unsalted crackers (Rakusen, Leeds, UK) during this break to cleanse their palate.

4.2.4 Statistical Analysis

All data analysis was conducted using XLSTAT (19.01, Addinsoft, New York, USA).

4.2.4.1 Physicochemical Analysis

Analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) post hoc test were conducted at $p<0.05$ for instrumental analysis. All analyses were conducted in duplicate across three sample bottles from the same batch, with an average mean calculated.

4.2.4.2 Quantitative Descriptive Analysis

A two factor ANOVA (sample, panellist) with interaction and Tukey's HSD post hoc test was performed on sensory results. A cluster analysis on mean scores of all sensory attributes was performed using agglomerative hierarchical clustering employing a dissimilarity matrix with Euclidean distance and Ward's method in the agglomeration (Yang et al., 2019).

4.2.4.3 Correlation between Physicochemical and QDA

Principle component analyses (PCA) were carried out on mean scores of physicochemical and sensory attributes to explore relationships. Both datasets used averaged scores across samples and only included sensory attributes and physicochemical results which significantly discriminated amongst the samples, as assessed by ANOVA. Sensory attributes were selected as one input matrix, with physicochemical analysis as supplementary variables.

4.2.4.4 Consumer Liking Analysis

To determine if differences existed amongst samples in terms of consumer overall liking a mixed model two-factor ANOVA (sample, consumer), with consumer as a random effect, was performed followed by Tukey's HSD post hoc test. A cluster analysis on the overall liking data was performed to see if liking patterns varied across consumers using agglomerative hierarchical clustering employing a dissimilarity matrix with Euclidean distance and Ward's method in the agglomeration. A correlation test (Pearson’s correlation coefficient) between each individual’s result and cluster mean was also performed to check the validity of cluster groups (Yang et al., 2019). Differences amongst samples within each cluster was explored through further analyses with a two-factor ANOVA. An internal preference map with PCA biplot of multivariate space of non-alcoholic lagers was also configured, using average overall liking scores of consumer clusters and QDA sensory attributes as supplementary variables, to better visualize the data and understand drivers of like and dislike for each cluster.

4.3 Results and Discussion

4.3.1 Physicochemical Analysis of Non-Alcoholic Beers

Instrumental analysis results for alcohol by volume (ABV), pH, bitterness units, total polyphenols and sugars can be found in Table 4.1. The ABV (%) of the NABs varied from 0.03 to 0.75 ABV. Although legal labelling criteria is different amongst countries, anything above 0.5% ABV cannot be classed as NAB (Department of Health & Social Care, 2018a), therefore samples 2 and 12 cannot be described as NAB. It is interesting to note that both these beers were produced by craft breweries, posing the question whether the correct controls are in place to measure the final product ethanol concentration before bottling. Differences amongst the beers in terms of ABVs were explored due to the different production methods used. It has been well documented that membrane based dealcoholisation processes are not economically viable to produce a beer <0.5% ABV (Pilipovik and Riverol, 2005) and therefore it is suggested here that samples 7, 8, 9 and 10 were produced through other physical methods, which may include spinning cone column. The majority of samples produced by vacuum distillation (12, 13 and 14) were shown to have the highest ABVs (0.75, 0.35 and 0.39 respectively), apart from sample 11 which had one of the lowest ABVs (0.03). It is believed that this trend could again be due to the economic feasibility of this process (Müller et al., 2017). Overall it seemed that there was variation in each of the production methods in terms of ethanol content, but generally dealcoholised beers had the lowest ABV, whilst beer produced by vacuum distillation had the highest. All beers had values within the scope of previously obtained results for pH, BU and TP for commercial beers, ranging from 3.99 to 4.81 for pH, 5.26 to 25.34 for BU

and 49.20 to 235.98mg/L for TP (Briggs et al., 2004). Samples 7 and 8 had the highest concentration of BU, and it is believed this is due to differences in starting hop concentrations amongst all the commercial beers. Sample 12 had the highest TP and this was the only sample that was unfiltered, so these polyphenols would not have been removed. The fermentable sugars measured were found to be higher in comparison to standard ABV beers (most notably for beers 1, 4, 5, and 8), which is proposed to be related to the production method used. Previous research has shown that biological production techniques produced beers with increased content of non-fermentable dextrins as the oligo- and polysaccharides in wort are not metabolized by yeast (Krebs et al., 2018). A clear differentiation in NABs produced by physical and biological methods due to differences in presence of sugars has been reported (Schmelzle et al., 2013), yet here it is shown that there are now products on the market which do not follow this rule. For example, samples 10 and 11 (produced by dealcoholisation and vacuum distillation) had higher maltose, and sample 2 (produced by altered brewing) had smaller amounts of this sugar, revealing that other factors influenced the presence of sugars.

HS-GC-FID analysis allowed identification of the most abundant compounds in beer, which included higher alcohols, esters and aldehydes (shown in Table 4.2). All compounds, except ethyl octanoate and ethyl decanoate, were significantly different amongst the eighteen samples ($p<0.05$). The volatile compounds identified varied amongst samples, showing that NABs have a broad range of flavour characteristics.

Table 4.2: *Concentration of most abundant volatile compounds and flavour thresholds in beer measured by HS-GC-FID for each sample. All flavour threshold values were stated based on literature from Morten C. Meilgaard (1982). Different letters within a column[abc] represent a significant difference amongst samples in terms of volatile concentrations (Tukey's HSD, $p<0.05$). Samples which have concentrations of volatile compounds greater than threshold are shown in bold. Samples with an asterisk (*) were those selected for the subset for consumer overall liking sensory analysis:*

Volatile Compounds (ppm)												
Sample Number	Production Method	**Acetaldehyde**	**Ethyl Acetate**	**Isobutyl Acetate (2-Methylpropyl Acetate)**	**Propan-1-ol**	**Ethyl Butanoate**	**2-Methylpropan-1-ol**	**Isoamyl Acetate**	**3-Methyl-1-Butanol**	**Ethyl Hexanoate**	**Ethyl Octanoate**	**Ethyl Decanoate**
1*	Altered Brewing	2.16^{cd}	5.29^{f}	0.00^{c}	0.00^{e}	0.00^{f}	0.11^{g}	0.67^{d}	8.36^{h}	0.05^{c}	0.03	0.00
2*	Altered Brewing	1.95^{cd}	2.08^{g}	0.00^{c}	7.70^{b}	0.00^{f}	3.03^{c}	0.12^{f}	17.68^{fg}	0.00^{h}	0.02	0.00
3*	Altered Brewing	3.76^{bcd}	0.00^{i}	0.00^{c}	0.00^{e}	0.00^{f}	0.00^{g}	0.00^{f}	0.00^{j}	0.00^{h}	0.00	0.01
4	Altered Brewing	8.76^{ab}	14.07^{c}	0.00^{c}	8.94^{a}	0.00^{f}	7.02^{a}	0.92^{c}	54.75^{b}	0.09^{b}	0.02	0.01
5*	Special Yeast	1.90^{cd}	0.00^{i}	0.00^{c}	0.00^{e}	0.00^{f}	0.00^{g}	0.00^{f}	0.00^{j}	0.00^{h}	0.00	0.02
6*	Special Yeast	9.16^{ab}	1.20^{gh}	0.00^{c}	5.30^{c}	0.00^{f}	4.81^{b}	0.07^{f}	33.17^{d}	0.00^{h}	0.01	0.00
7*	Dealcoholised	**11.74^{a}**	0.12^{i}	0.00^{c}	0.00^{e}	0.00^{f}	0.00^{g}	0.00^{f}	0.00^{j}	0.00^{h}	0.00	0.02
8	Dealcoholised	1.47^{cd}	0.00^{i}	0.00^{c}	0.00^{e}	0.00^{f}	0.00^{g}	0.00^{f}	0.00^{j}	0.00^{h}	0.00	0.02

9	Dealcoholised	0.77^{d}	**31.95^{a}**	0.00^{c}	0.00^{e}	0.01^{def}	0.00^{g}	**4.24^{b}**	42.55^{c}	0.03^{def}	0.00	0.01
10	Dealcoholised	1.59^{cd}	0.20^{I}	0.02^{b}	0.00^{e}	0.02^{cd}	2.20^{e}	0.43^{e}	34.79^{d}	0.05^{cd}	0.02	0.03
11	Vacuum Distillation	4.05^{bcd}	0.40^{hI}	0.02^{b}	0.00^{e}	0.02^{cd}	0.14^{g}	0.99^{c}	7.73^{h}	0.02^{efg}	0.00	0.02
12*	Vacuum Distillation	6.12^{abcd}	5.24^{f}	0.00^{c}	2.97^{d}	0.01^{ef}	3.28^{c}	0.39^{e}	21.06^{ef}	0.02^{fgh}	0.01	0.00
13*	Vacuum Distillation	1.45^{cd}	8.72^{d}	0.08^{b}	0.85^{e}	0.05^{a}	2.56^{de}	0.68^{d}	13.95^{g}	0.02^{fg}	0.03	0.00
14	Vacuum Distillation	**11.62^{a}**	6.96^{e}	0.17^{a}	3.05^{d}	0.02^{cde}	2.94^{cd}	0.61^{d}	24.23^{e}	0.04^{cde}	0.02	0.01
15*	Mixed Methods	4.26^{bcd}	1.56^{g}	0.00^{c}	3.46^{d}	0.00^{f}	1.38^{f}	0.11^{f}	6.77^{hI}	0.01^{gh}	000	0.00
16*	Mixed Methods	7.91^{abc}	16.01^{b}	0.00^{c}	0.00^{e}	0.02^{cde}	0.00^{g}	**6.18^{a}**	**93.97^{a}**	0.14^{a}	0.02	0.02
17*	Mixed Methods	3.45^{bcd}	1.41^{g}	0.00^{c}	0.00^{e}	0.04^{ab}	0.00^{g}	0.33^{e}	6.98^{hI}	0.03^{def}	0.00	0.00
18	Mixed Methods	1.55^{cd}	0.02^{I}	0.00^{c}	0.25^{e}	0.03^{bc}	0.00^{g}	0.34^{e}	2.94^{Ij}	0.03^{cdef}	0.00	0.02
P Value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.019	0.506
Flavour Threshold in Beer (ppm)		10	30	1.60	800	0.4	200	1.2	70	0.21	0.9	1.5

The presence or absence of these compounds was explored in relation to production methods. In terms of higher alcohols, sample 16 was found to have increased levels of 3-methyl-1-butanol (93.97mg/L), followed by sample 4 (54.75mg/L) compared to other beers. Samples 2 and 4 had increased amounts of 2-methylpropan-1-ol and propan-1-ol. Higher alcohols are the precursors to most flavour active esters, therefore when fermentation is halted prematurely in the brewing process these higher alcohols do not have sufficient time to be converted into esters (Briggs et al., 2004). Thus these samples were also found to have significantly reduced amounts of ethyl acetate and isoamyl acetate. Samples 7 and 8 (produced by dealcoholisation) were found to have none of these higher alcohols, agreeing with previous research that dealcoholisation removed a large amount of these important volatiles due to similarities with ethanol in terms of boiling point or molecular size (Müller et al., 2017). In terms of esters, samples 4, 9 and 16 (all produced using different production methods) had increased levels of ethyl acetate in comparison to other samples. Samples 9 and 16 also had increased amounts of isoamyl acetate. It is believed that these samples had higher levels of these esters due to either the addition of natural flavourings, or due to current advances in technologies. One example of this is the capturing of flavour concentrates from dealcoholized beer through pervaporation, which can then be blended back with the beer to increase the flavour profile to that of a standard beer (Branyik et al., 2012). Beers produced by altered brewing (1, 2 and 4), had significantly more ethyl acetate than those produced by physical methods (7, 8 and 10). Acetaldehyde is also a key volatile in beer, which is often discussed as an ‘off-flavour ’which arises from oxidation (Briggs et al., 2004). Samples 7 and 14

contained the highest amount of this volatile compound and it is believed that this was due to poor bottling technique, increasing oxygen levels and leading to contamination from spoilage microorganisms (Sohrabvandi et al., 2010). Other reasons for these increased levels include poor yeast health, excessive wort oxygenation, high fermentation temperatures, excessive pitching rates or lack of fermentation vigour (Briggs et al., 2004). Interestingly, it was thought that the beers produced by craft breweries may contain more of these 'off-notes' due to limited capabilities of quality control measures, but all samples produced by craft breweries (2, 12, 13 and 15) had lower amounts. These physicochemical measurements suggest that there are many factors influencing the presence and quantity of flavour compounds of NABs, which not only include NAB production method but also pre and post processing methods.

4.3.2 Descriptive Sensory Analysis of Non-Alcoholic Beers

Mean attribute scores and results from significance testing were calculated for all eighteen commercial NABs, using QDA with the trained panel (data not shown, Appendix Table 4). ANOVA revealed that for all twenty-three attributes, significant product differences were found ($p<0.0001$). The data was clearly visualised by the use of a PCA (Figure 4.2), showing the multivariate space of the NABs and their sensory attributes. The first two principal components (PCs) of the model accounted for 69.02% of variation in the data (36.53% and 32.49% for PC1 and PC2, respectively). PC1 was strongly positively correlated to cooked vegetables (0.817), rubbery (0.882), sulphur (0.925) and burnt (0.890) aromas, initial (0.806) and lingering (0.806) bitterness, cardboard flavour (0.756), metallic (0.941) and astringent (0.790). PC1 was negatively correlated with floral aroma (-0.696) and sweet (-0.620).

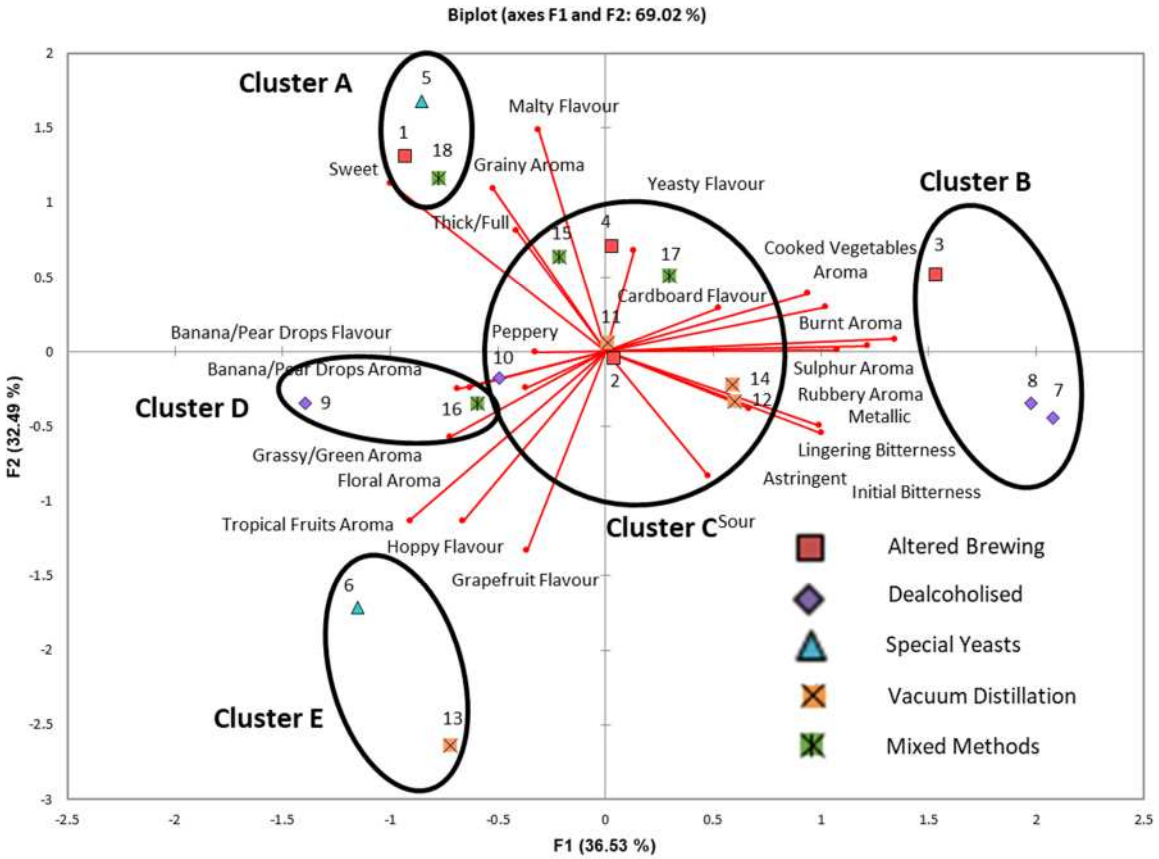


Figure 4.2: *Principal component analysis (PCA) biplot of significant attributes present on principle component 1 and 2 by the covariance of mean significant attribute intensity ratings across non-alcoholic commercial lager samples with different production methods. Clusters of samples with similar sensory attributes, analysed using agglomerative hierarchical clustering, are circled and labelled in black.*

PC2 was strongly correlated with grainy aroma (0.756), thick/full (0.805), sweet (0.729), malty (0.894) and yeasty (0.822) flavour and negatively correlated with tropical fruits aroma (-0.694), grapefruit (-0.819) and hoppy (0-0.668) flavour and sour (-0.805). PC3 (not shown) explained 15.88% of variance in the data and this is due to being strongly correlated with banana pear drops aroma (0.850) and flavour (0.879) and peppery (0.680) and negatively correlated with hoppy flavour (-0.549).

Mean attribute scores were also subjected to cluster analysis (Figure 4.2, dendogram shown in Appendix Figure 2) to determine whether distinct subgroups of NABs could be identified and clusters explained by production method. Five clusters were easily identifiable. Cluster A (1, 5 and 18) contained samples which were positively correlated to grainy aroma, malty flavour, sweet and thick/full and all were produced by different methods. It should be highlighted that the term 'malty flavour' used here included worty characteristics. During panel training, panellists recognised many of the samples had a 'worty 'characteristic, confirmed through the use of a wort sample as a reference, however the descriptor 'malty flavour' was selected by the panel (see Appendix Table 3). Cluster B (3, 7 and 8) contained samples correlated to cooked vegetable, sulphur and rubbery aromas, initial and lingering bitterness, metallic and astringent aftertastes. Samples 7 and 8 were made using dealcoholisation techniques, with no additional adjuncts, however sample 3 was made using altered brewing techniques with the addition of the adjunct corn (Table 4.1). This may explain the strong correlation with the attribute 'cooked vegetable aroma' for this sample. It may also help to explain why it is clustered with samples made using physical processes as these

methods have been previously associated with the above attributes (Schmelzle et al., 2013). Cluster C, the largest cluster in this sample set (2, 4, 10, 11, 12, 14, 15 and 17) contained products not well described, receiving ratings close to the mean of the attributes, showing that these beers had a rather bland flavour profile. Cluster D contained samples (9 and 16) which were associated with banana pear drops aroma and flavour. These samples were found to contain 'natural flavourings', which may explain the banana/pear drop aroma and flavour characteristics. Peppery was an attribute that was discovered in sample 9 only, and this was in reference to the perception of heat/chilli. Previous research has looked at the effect of different irritants on their pungency using descriptive analysis (Cliff and Heymann, 1992) and found that ethanol brought burning and tingling sensations, with other irritants showing similar properties. It is therefore hypothesised that the commercial brewer for this sample could have introduced a similar irritant to counteract the lack of these sensations. However, common irritants such as eugenol, cinnamaldehyde and 4-vinylguaiacol (Cliff and Heymann, 1992, Lentz, 2018) were not found in GC-MS analysis. Cluster E (6 and 13) were found to have a hoppy aroma with high correlations to descriptors such as tropical fruits and floral aroma. It is believed that this was due to the samples being subjected to post-processing methods, such as dry hopping resulting in these aromas being perceived by the panel. Sample 13, was confirmed to be dry-hopped after the process.

It has previously been suggested that the production method used is the main factor for the differences in sensorial profiles of NAB (Schmelzle et al., 2013), yet interestingly here this factor was not found to be the main driver of membership of beers within these clusters. Indeed, if this study had only

categorised the samples into ‘biological and mixed methods ’and ‘physical ’ production processes it would have shown a similar trend to that shown previously (Schmelzle et al., 2013), whereby biological methods produced malty, worty and sweet beers, and physical methods produced bitter, sour and sulphur-like beers. However, here it appears that the sensory differences were due to other factors, such as pre and post processing methods, which reflects the increased development in this sector resulting in NABs with more complex sensorial profiles.

4.3.3 Correlation between physicochemical and descriptive sensory analysis results

Combining physicochemical and sensory results provides a comprehensive characterization of NABs. The correlation circle (as shown in figure 4.3a), shows sensory attributes with physicochemical results overlaid as supplementary data (further information on SPME-GC-MS data can be found in the Appendix, Table 5). As expected, attributes such as banana/pear drops aroma and flavour were projected similarly to volatile compounds well known for these attributes in beer; isoamyl acetate and 3-methyl-1-butanol. Fermentable sugars were also projected similarly with sensory attributes such as malty flavour, sweet and thick/full (Bellut and Arendt, 2019). An interaction between sweet and thickness/full attributes in the QDA analysis revealed that all samples rated higher in terms of sweetness were also rated higher for thick/full.

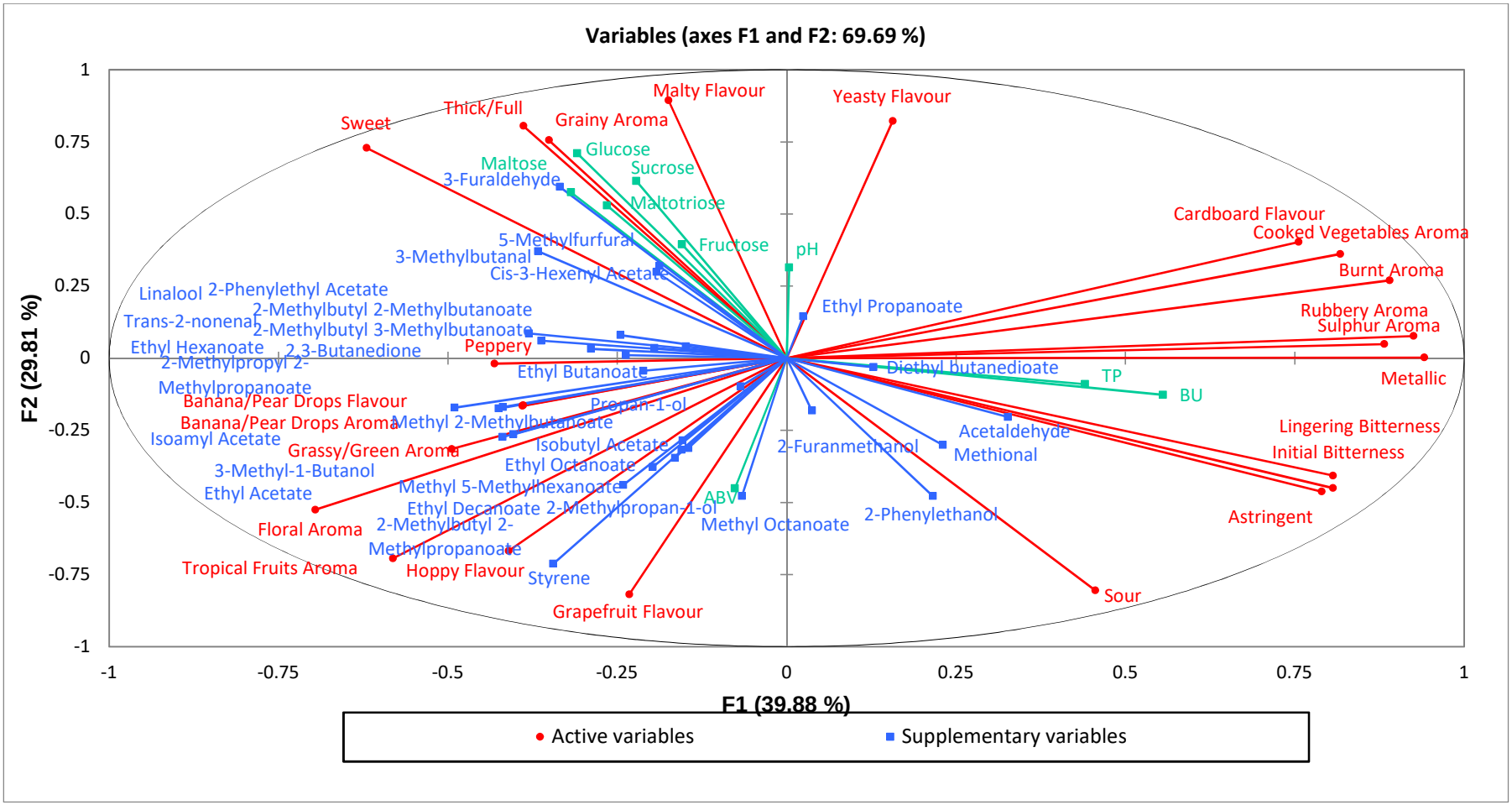


***Figure 4.3a**: Correlation biplot of all physicochemical and sensory data showing significant attributes present on principle component 1 and 2. Attributes in red show QDA sensory attributes, those in blue show volatile compounds found through GC-MS and those in green show all other instrumental analysis.*

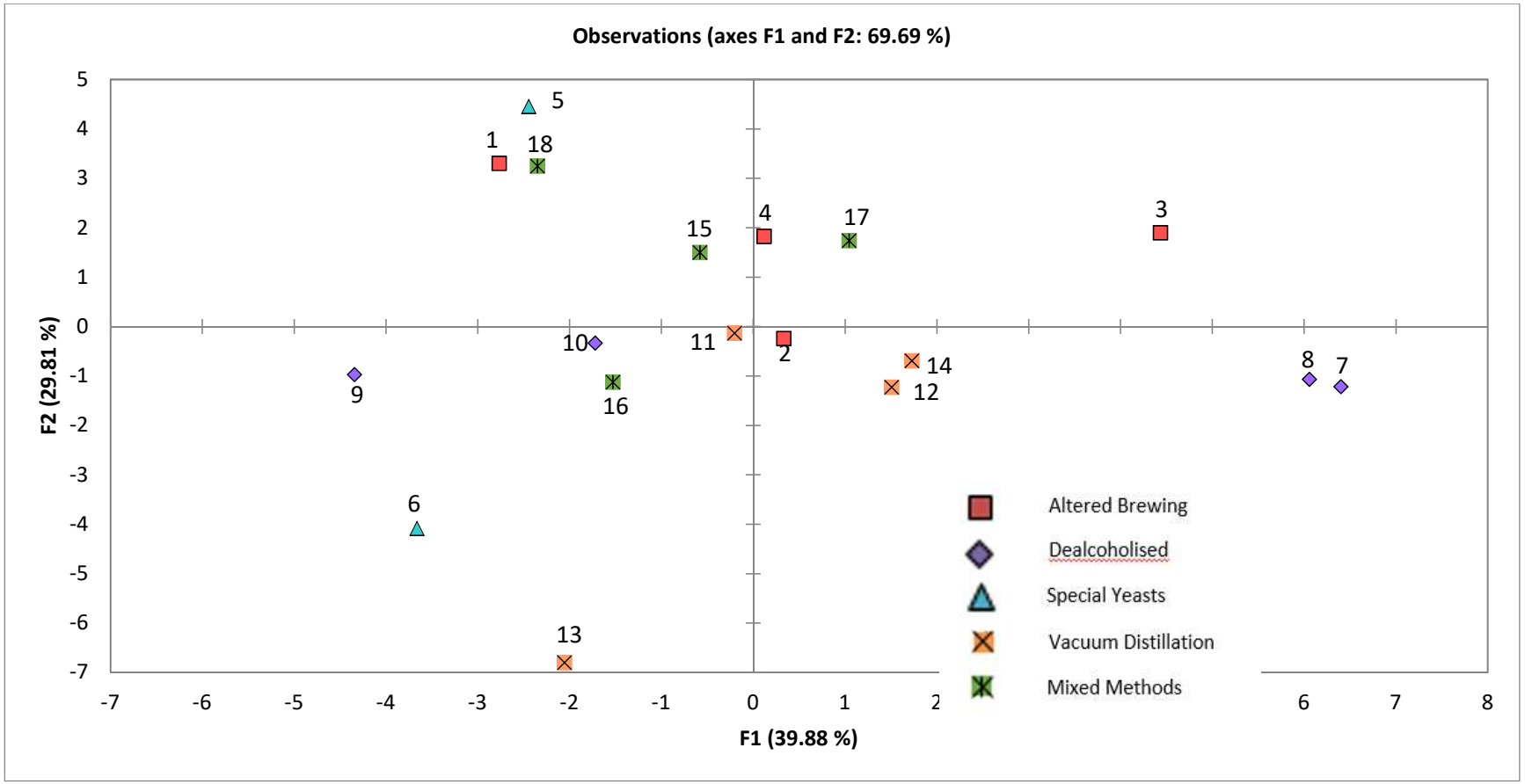


Figure 4.3b: *Principal component analysis (PCA) biplot of samples present on principle component 1 and 2 by the covariance of mean significant attribute intensity ratings by QDA and mean of instrumental analysis across non-alcoholic commercial lager samples with different production methods*

Total polyphenol content and bitterness units were correlated to initial and lingering bitterness, as well as astringency, with previous literature suggesting these physicochemical aspects relate to their sensory properties (Oladokun et al., 2016). Interestingly, no compounds were identified to correlate to the attributes of cooked vegetable, burnt, sulphur and rubbery aroma, cardboard flavour or metallic. However, this may be due to the presence of highly odour active compounds at very low concentrations, such as sulfur compounds (dimethyl sulfide (DMS), dimethyl disulfide, dimethyl trisulfide, sulfur dioxide) which were not identified in the GC-MS analysis because the method was not sensitive or selective enough to identify them. Further work utilising a flame photometric detector (FPD) (Mundy, 1991) or sulfur chemiluminescence detection (SCD) (Burmeister et al., 1992) is therefore suggested to understand the presence of sulfur compounds in NABs and their contribution to these attributes.

Overall the 18 samples were found in different locations of the PCA plot (as shown in figure 4.3b), reflecting the distinctive physicochemical and sensorial properties amongst the samples. PC1 was not correlated with any of the physicochemical data, yet samples 3, 7 and 8 (cluster B) were all positively correlated with this PC. Samples 7 and 8, had cooked vegetable aroma, sour and bitter tastes, with previous studies finding similar results and correlating this to the presence of DMS (Müller et al., 2017). Interestingly, it has been proposed that these 'off-flavours', as well as bitterness, become more dominant if other volatile compounds are removed to below threshold level, meaning the synergistic effects of the overall beer flavour become unbalanced (Gernat et al., 2019, Müller et al., 2017). This appears to be the case for these two samples, as

they were only found to contain acetaldehyde in the lower boiling point volatile analysis. In addition to this, these samples were found to have decreased thick/full sensory ratings.

PC2 was strongly correlated with glucose (0.710), sucrose (0.614), maltose (0.575), maltotriose (0.529) and furfural (0.594). Samples 1, 5 and 18 (Cluster A) were situated close together and were positively correlated with PC2, with a grainy aroma, malty flavour, sweet and thick/full. These samples had increased levels of fermentable sugars, as well as 3-methylbutanal and furfural. Previous literature found that many factors can enhance the perception of undesirable sensory characteristics of 'worty 'and 'potato-like ' in beers, including; presence of significant amounts of aldehydes (furfural, 2-methylbutanal, 3-methylbutanal and 3-methylthiopropionaldehyde) (Perpete and Collin, 2000), absence of higher alcohols and esters which have been found to help mask these off-flavours (Saison et al., 2009) and the presence of increased amounts of fermentable sugars (Perpete and Collin, 2000).

PC2 was negatively correlated with styrene (-0.713). Samples 6 and 13 were strongly correlated with this PC (Cluster E). The presence of styrene within these beer was a surprising finding, especially as it was found in its highest quantities in the craft beers, however the origin of this was unknown. They both had a sensorial profile of tropical fruits and floral aroma, grapefruit and hoppy flavour and sourness which is likely to be due to the dry hopping technique employed for sample 13, and proposed here for sample 6 (although unconfirmed). Previous research has looked at increasing aroma intensity of low alcohol beer (1.2-1.4% ABV) by late hopping, and showed similar results to the current study of more intense fruit, citrus-like, green-grassy, and hop-

spicy odour notes (Forster and Gahr, 2011) whilst also disguising off-flavours (such as styrene) by masking effects to improve overall aroma impression (Müller et al., 2017).

PC3 (data not shown here, see Appendix Figure 3) was strongly correlated with ethyl acetate (0.529) and isoamyl acetate (0.687) and negatively correlated with 3-methylbutanal (-0.526) and methyl 2-methylbutanoate (-0.625). Samples 9, 10 and 16 were correlated with this PC (Cluster D). Sample 9 contained ethyl acetate and isoamyl acetate above threshold (31.94mg/L and 4.24mg/L respectively), and sample 16 also contained isoamyl acetate above threshold.

Finally, samples 2, 4, 11, 12, 14, 15 and 17 (Cluster C) were found to all be close to the centre of the PCA biplot, with similar means for all attributes. Samples 11, 12 and 14 in particular appear to be lacking volatiles which agrees with the lack of specific sensory characteristics defining them.

Whilst the physicochemical and sensory data showed that resulting profiles did not appear to be related to production method when explored separately, when looking at this data together, some broad learnings appear. When comparing dealcoholized beers to those produced using biological methods, biological methods were found to have increased body. It is believed that this is due to brewers using a stepped mash profile, which consists of altering temperatures and timings to improve the body and mouthfeel of NABs (Branyik et al., 2012). Conversely, samples with decreased thickness/fullness were found to follow previous literature that states that beers produced using physical methods have less body (Montanari et al., 2009). Samples 1 and 5

were both produced by biological production methods, with 5 being one of only two samples produced by special yeasts, and showing similar profiles of beer produced via this method to previous literature (Bellut and Arendt, 2019). Although the particular yeast strain used in this beer cannot be confirmed, previous research has suggested that *Saccharomyces ludwigii* is the most successful commercially available low alcohol yeast, used for industrial production (Branyik et al., 2012). It appears that all samples produced using vacuum distillation (12, 13, 14) were lacking in volatiles and dominant sensory attributes. Therefore, it seemed that this method removed a significant amount of volatiles, supporting previous literature which showed 76-97% of esters and 88-95% higher alcohols can be removed, due to similar boiling points to ethanol (Montanari et al., 2009). Interestingly, samples produced by this method had increased levels of 2-furanmethanol, which is a compound that serves as a marker for the heat load impact on the beer; in this case showing a small, but indeed relevant, heat-induced off-flavour (Gernat et al., 2019).

On the other hand, there are some samples which clearly did not follow a trend in relation to their production method. Samples 7,8, 9 and 10 were all dealcoholised beers and whilst samples 7 and 8 followed previous literature with regards to their sensory properties, samples 9 and 10 showed completely different profiles. Samples 9 and 10 were shown to have 'natural flavourings' added to the ingredients list, suggesting this to be the cause. Interestingly, sample 3 (produced through interrupted fermentation) gave a similar sensory and physicochemical profile to samples 7 and 8 (produced by dealcoholisation methods). There is no clear explanation as to why this was the case, however it could be due to lack of vigour in the fermentation vessel during production,

meaning that other compounds such as esters were not able to develop to mask these 'off flavours' (Saison et al., 2009).

Therefore, the current data shows that the variation in sensory and physicochemical profiles of NABs may not only be due to the production methods used but also attributable to other important factors including different starting raw materials (such as the addition of adjuncts including rye, wheat, rice, corn or maize) or post processing methods (such as the use of additive flavour compounds, dry hopping or addition of liquid hop products post fermentation). One limitation of this study was that these beers were commercially produced, therefore it is difficult to draw conclusions on the real impact of production methods and pre and post processing methods on the sensory characteristics of these beers. It does however, show that there are a wide range of NABs with different sensory profiles on the current market, and the flavour profile of different production methods can be varied utilising different raw materials or post processing methods.

4.3.4 Consumer Liking Analysis

One of the key interests for the brewing industry is to understand the most desired flavour profile by consumers for a NAB. This was explored through the use of a consumer panel registering their overall liking of a subset (n=11) of the eighteen samples selected from QDA for their range of flavour characteristics. In the initial analysis of overall liking for the eleven selected samples, significant differences were found ($F(10, 1143) = 6.874$, $p=<0.0001$), with samples 15 and 17most liked (mean= 6.221, SD= 1.393 and 1.966 respectively). These samples were both found to be in Cluster C, which were previously proposed to be perceived as having a bland flavour profile, with

none of the sensory attributes rated highly for these samples. These samples were produced via mixed methods, indicating that overall liking for consumers could be optimised by mixing different production techniques. The samples that were least liked were samples 2 (mean= 5.058, SD= 2.189) and 7 (mean= 4.740, SD= 1.900), which were found to have significantly higher initial and lingering bitterness, as well as astringency. Subsequent application of agglomerative hierarchical clustering (AHC) analysis was performed to identify different clusters of consumers within the data set.

Figure 4.4 shows PCA mapping of five consumer clusters identified. The ANOVA yielded significant differences for the interaction between sample and cluster ($F(4, 1143) = 7.901$, $p=<0.0001$), indicating that the overall liking of the samples varied with each consumer cluster. The first two principal components (PCs) of the model accounted for 73.69% of variation in the data (39.79% and 33.90% for PC1 and PC2, respectively). PC1 was strongly positively correlated to C1 (0.531), C3 (0.668), C4 (0.756), thick/full (0.523), sweet (0.546) and malty flavour (0.618). PC1 was negatively correlated to C5 (-0.716), initial (-0.538) and lingering (-0.530) bitterness, grapefruit (-0.647) and hoppy (-0.558) flavours, sour (-0.582) and astringent (-0.588).

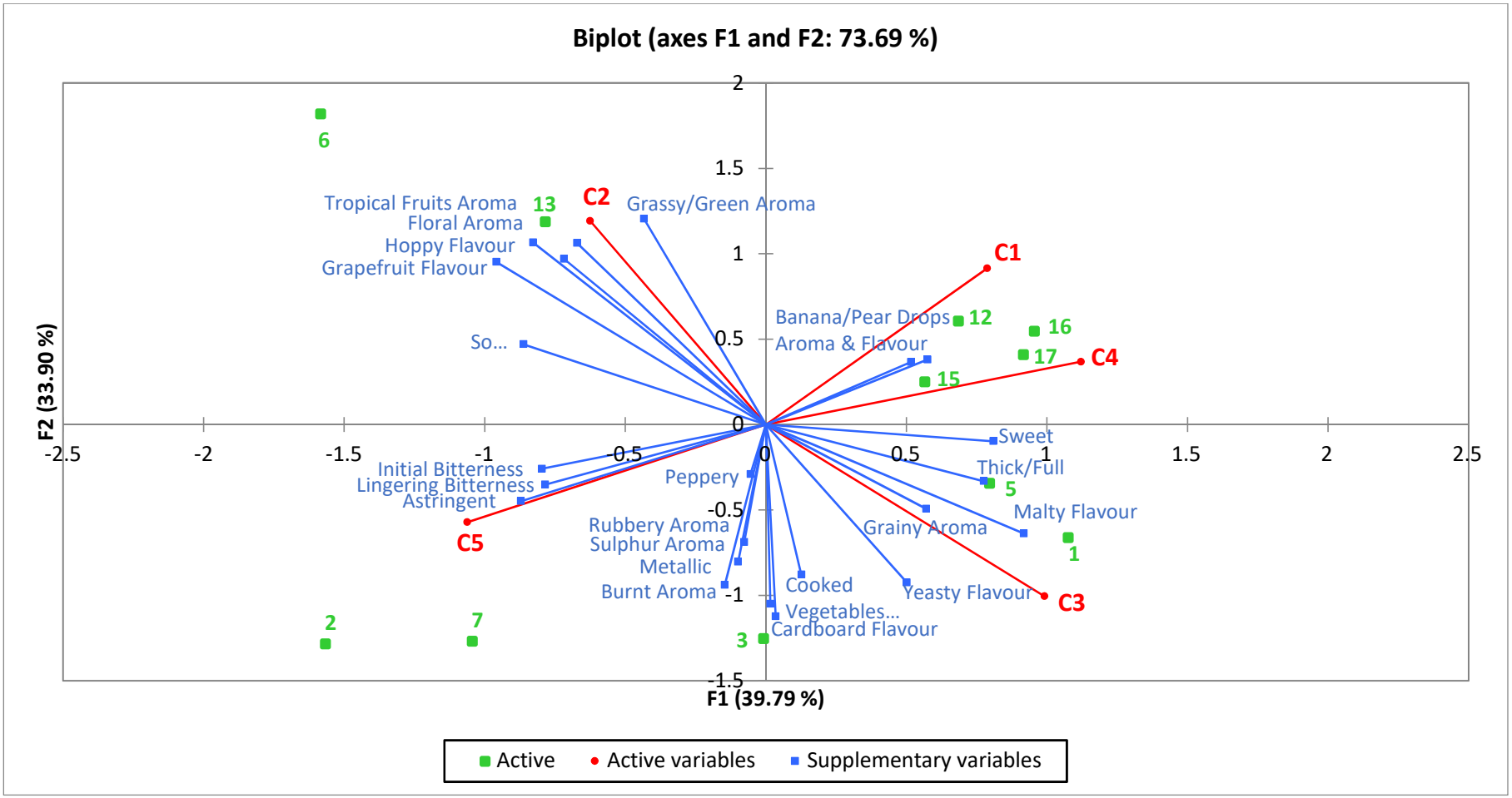


Figure 4.4: *Internal preference map of mean overall liking data per cluster, with QDA sensory attributes as supplementary data.*

Red shows cluster number, green shows sample number and blue shows QDA sensory attributes.

PC2 was strongly positively correlated with C1 (0.617), C2 (0.804), grassy/green (0.813), tropical fruits (0.717) and floral (0.654) aromas, grapefruit (0.642) and hoppy (0.719) flavours. PC2 was negatively correlated with C3 (-0.677), burnt aroma (-0.708), cardboard (-0.757) and yeasty (-0.623) flavours and metallic (-0.633). Statistically, scores for cluster 1 (C1, n=28) showed differences for consumers liking ($F(10, 307)=10.027$, $p=<0.0001$) with Tukey's HSD test indicating the overall liking was lowest for samples 2 and 7 ($p=<0.0001$). These consumers were described as 'bitter dislikers', as they were positively correlated to PC1. This was negatively correlated with attributes initial and lingering bitterness and astringent, with these consumers disliking the samples which were rated highest for these attributes. Cluster 2 (C2, n=28) yielded differences amongst samples ($F (10, 307) = 16.073$, $p=<0.0001$) and showed consumers within this cluster liked samples 6 and 13 and disliked samples 1 and 5. These consumers were described as 'hoppy likers', as this cluster was positively correlated with PC2, which was in turn positively correlated to hoppy and grapefruit flavours. The samples they most liked were those that had been dry hopped and were also described as hoppy by the QDA panel. C3 (C3, n=12) were found to like samples ($F (10, 131) = 6.985$, $p=<0.0001$) 1, 3, 16 and 17, and dislike samples 6 and 13, showing the opposite of C2. This was confirmed by a negative correlation to PC2 and therefore these consumers were described as 'hoppy dislikers'. In a study of Brazilian beer consumers, it was found that the least preferred beer style in the sample set was India Pale Ale and this was linked to the samples being hop-forward with increased bitterness, as well as having a characteristic floral note (Jardim et al., 2018), which was also found with samples 6 and 13 here. This

could therefore explain why the consumers in this cluster did not like these samples. C4 (C4, n=17) liked samples 15, 12, 17, 1 and 13 the most (F (10, 186) = 9.537, p=<0.0001) and sample 2 the least. This cluster was positively correlated to PC1, which was positively correlated with thick/full, sweet and malty flavour and thus these were described as 'malty/sweet likers'. Previous research (Porretta and Donadini, 2008) has shown that overall preference is highest for a full bodied beer with a malty and sweet taste, and consumers within this cluster seemed to follow this trend. C5 (C5, n=19) showed no difference in overall liking amongst the samples (F (10, 208) = 0.872, p=0.560) and rated all samples as 'like slightly'. Although this cluster was negatively correlated with PC1 and thus correlated with bitterness and astringency, consumers within this cluster showed no clear preference for any of the samples. Therefore they were described as 'enthusiasts', as their overall liking for all samples was higher than other clusters; a similar group was found in beers with different ethanol concentrations (Ramsey et al., 2018) and bread (Gellynck et al., 2009).

The present study showed that there are key differences within a population for NAB liking, confirmed due to the large number of clusters, which has also been found for standard beers (Guinard et al., 2001) and is a key finding for the brewing industry. When data was analysed at surface level, the most liked samples were those with a fairly bland flavour profile. Yet when clustering was applied, it became apparent that samples with strong flavour profiles are either enthusiastically liked or disliked, shown by clusters of 'hoppy likers' and 'hoppy dislikers'. This suggests that in the NAB sector, no one size fits all and therefore a company could be missing key insights by

only looking at the mean data. Furthermore, this data shows that a variety of NABs with different sensory profiles are required to satisfy different consumer groups.

Finally, the overall liking score range amongst all clusters was found to be narrow, with consumers citing that they ‘slightly liked ’or ‘slightly disliked ’ samples, and this was similar to ranges found by Ramsey et al. (2018) in terms of 0% beer. Therefore this shows that improvements are still required in this product space to ensure consumers are provided with sensorially acceptable products. On the other hand, consumers did not strongly oppose any of the beers, so good progress in the sensory quality of NABs is being made. It is important to note that the number of consumers per cluster were too low to draw strong conclusions so results for each cluster can only be viewed as trends in consumer data. Suggestions for future work are therefore to replicate the study with a larger group of consumers to understand the robustness of consumer cluster trends. These results could be used to advance the understanding of consumer liking of NAB.

Overall, this study provides a greater understanding into the differences between commercial NAB using physicochemical and sensorial techniques, and highlights that pre and post production methods should be taken into consideration when exploring relationships with production method. Advancements in new technologies have seen increased product development in this sector, with this research providing insight into the consumer demand for a wide range of sensory characteristics for NABs.

4.4 Conclusion

This study used instrumental techniques, a trained sensory panel and a consumer panel to evaluate the differences in commercial NAB in terms of physicochemical properties, perception of sensory attributes and their influence on consumer liking. Overall it showed that there is a clear range of non-alcoholic lagers currently on the market in the EU, as breweries increased development to satisfy increased consumer demand. Advances and improvements in pre and post processing methods and production techniques were also shown. Contrary to previous findings revealing that production methods are the main factor in altering the physicochemical and sensory properties of NAB, this study showed many exceptions due to the use of mixed methods and pre and post-production practices. It therefore poses the question whether pre-processing factors (such as raw materials used) or post-brewing processes (such as the use of additive flavour compounds or dry hopping) have more of an influence on the overall quality of NAB. These therefore may be utilised by breweries to produce a wide range of NAB with different sensory profiles that are liked by different consumer clusters. In terms of overall liking, five different clusters of consumers were found, showing different liking trends and therefore key differences within the population.

This research is important for the global brewing industry as it gives valuable insight regarding the sensory impact of pre and post processing methods on the development of new NABs. Brewers can use this as a guide to select their desired NAB sensory characteristics, helping to fill a void in their current repertoire. Altering the sensorial profile of NAB in this way could be

valuable to smaller craft breweries who may not have the capabilities to purchase expensive dealcoholisation equipment.

5 Assessing the sensory and physicochemical impact of reverse osmosis membrane technology to dealcoholize two different beer styles

Preliminary thoughts Chapter 5:

Chapter 4 found that the least liked NAB by consumers were those with higher bitterness and astringency ratings, which previously have been found to be related to physical dealcoholisation methods. Sample 7, one of the most disliked samples, was made using a physical dealcoholisation method showing that improvements are clearly needed for physical production methods.

Membrane separation techniques, such as reverse osmosis (RO) and nanofiltration (NF), were highlighted as promising physical processing methods to produce NAB in the literature reviewed in Chapter 1. These processes use a selective membrane to separate ethanol from the product matrix. RO has been used in previous studies to dealcoholize beer with minimal losses of volatiles, however there is no literature exploring the impact

of this technique on sensorial properties. In addition, NF has only been reported in the dealcoholisation of wine, yet was highlighted as a potential method to dealcoholize beer.

Interestingly, only a handful of studies have reported the changes in chemical and physical parameters of the resulting beers by recording aroma content, colour, bitterness, pH, total phenolic compounds and antioxidant activity. Few have reported on optimisation of processing parameters such as membrane selection, operating pressures and temperatures. None have reported on the effect of membrane processing on different product matrixes, or changes on sensorial characteristics after dealcoholisation. Therefore the aim of the following study was to fill the void in current literature, by assessing the impact of RO on the physicochemical and sensory properties of different beer styles, to understand the efficacy of this method for producing lower alcohol versions of standard beers.

Before the work conducted in this chapter commenced, parameters exploring the most efficient membrane type, operating temperatures and pressures of the dealcoholisation unit were conducted to optimise ethanol reduction, whilst maintaining volatiles and operating costs. An overview of these preliminary tests are reported here, with the methods and results presented in the format of an extended abstract.

5.1 Assessment of different membrane technologies and processing parameters on beer flavour using a pilot scale dealcoholisation unit

A pilot scale dealcoholisation unit, LabStak M20-0.72 was fitted with spiral wound membranes for the first trials (Reverse Osmosis membranes - RO99 (99% rejection measured on 2000ppm NaCl), RO90 (90% rejection measured on 2000ppm NaCl) and Nanofiltration membrane NF (99% rejection on 2000ppm magnesium sulphate)), all made up of a thin film composite polyamide membrane with polyester support material, measuring 1.9m². Ethanol content was measured using an Anton Paar Alcolyzer and lower boiling point beer volatiles (ethyl acetate, 1-propanol, isobutanol, isoamyl acetate, 3-methyl-1-butanol, ethyl hexanoate, ethyl octanoate) were measured using Headspace Gas Chromatography-Flame Ionization Detector (HS-GC-FID) following the method reported in chapter 4. Operating parameters were selected from manufacturers' guidance (20 and 30 bar pressure, 10 and 20 ºC temperature). Analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) post hoc test were conducted at $p<0.05$. The time taken to reach a reduction in ethanol content compared to the original beer was recorded for different trials, and a percentage change was considered for all instrumental analysis at this time point. All analyses were conducted in triplicate, with an average mean calculated.

In the first trials comparing different membranes and their composition (RO99, RO90 and NF), results showed that the NF membrane was the most efficient at ethanol reduction (Figure 5.1). The time taken to reach a 75% reduction in ethanol concentration was 170 mins, half the time taken for RO99.

The RO90 membrane took 230 mins, and thus was the middle ground membrane.

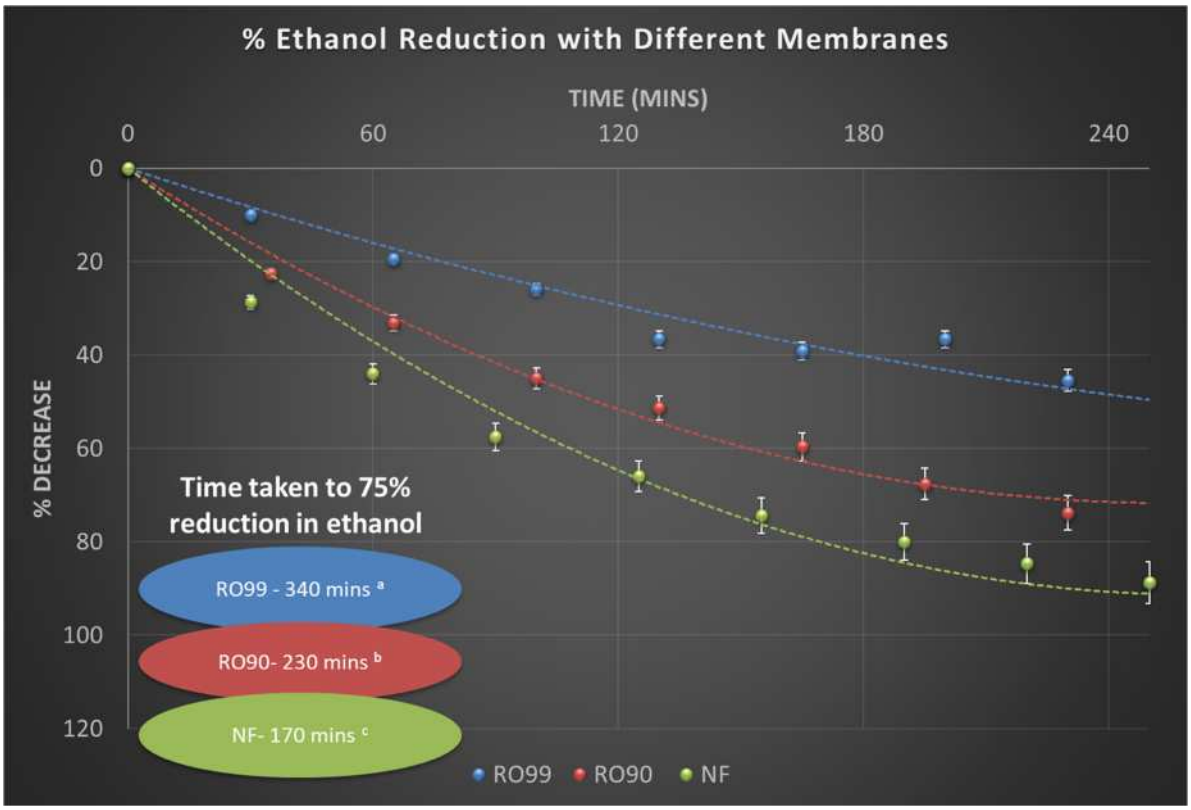


Figure 5.1: *% decrease of ethanol concentration over time using different membranes; RO99 (blue), RO90 (red) and NF (green). Time taken to reach a 75% reduction in ethanol concentration also shown. Different letters* ^{abc} *between trials represents a significant difference (Tukey's HSD, $p<0.05$).*

However, this was at the detriment of key volatiles in beer, with the NF membrane removing around 60% of higher alcohols (isobutanol and 3-methyl-1-butanol) and 30% of esters (ethyl acetate and isoamyl acetate). RO99 was found to be the most selective membrane, removing only 20% of higher alcohols and esters, whilst the RO90 membrane removed around 25% esters and 30% of higher alcohols. Considering the need for efficient ethanol removal

whilst retaining volatiles, the RO90 membrane was selected for the proceeding trials exploring operating temperatures and pressures.

Results (figure 5.2) found that higher pressure (30 bar) and higher temperature (20 °C) were the most efficient at the removal of ethanol. In terms of changes to volatile composition, higher pressures (30 bar) removed greater amounts of higher alcohols (25% 1-propanol, 5% isobutanol) and esters (55% ethyl acetate and 18% isoamyl acetate) compared to the lower pressure (20 bar).

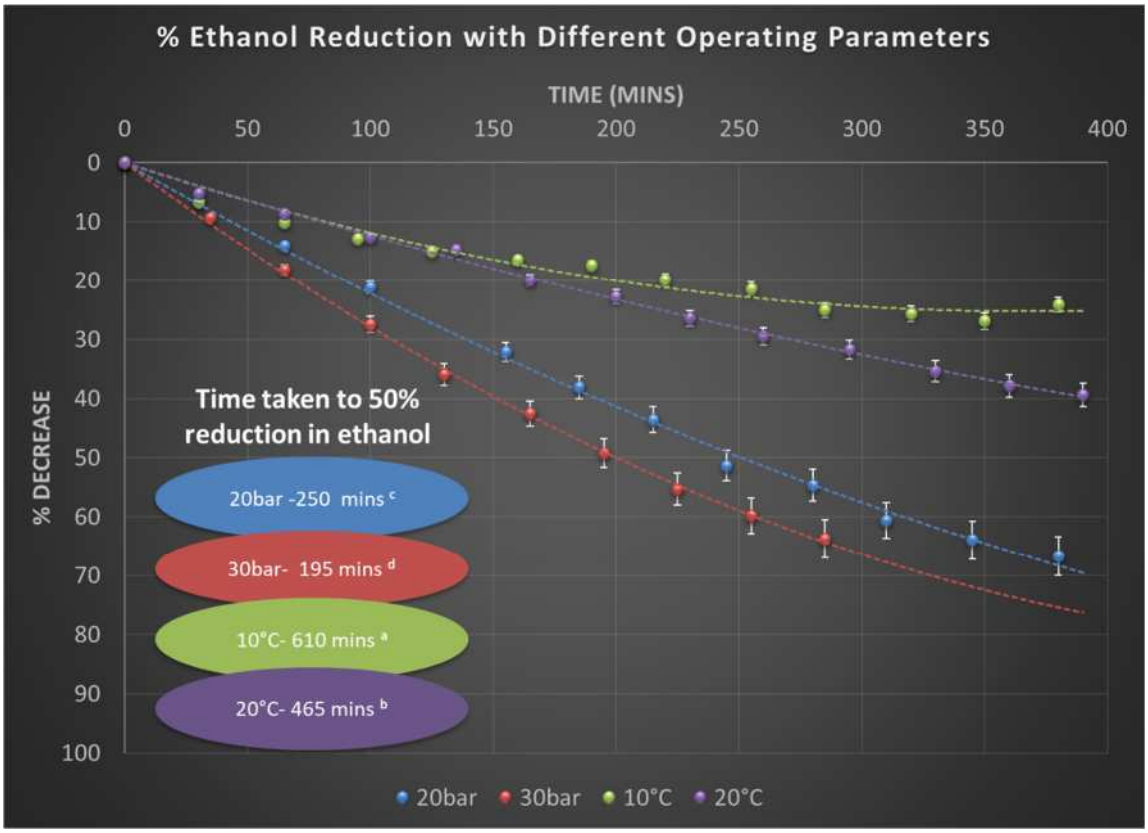


Figure 5.2: *% decrease of ethanol concentration over time using different operating parameters; pressures – 20 bar (blue), 30 bar (orange), temperatures – 10 °C (grey) and 20 °C (yellow). Time taken to reach a 50%*

reduction in ethanol concentration also shown. Different letters[abcd] between trials represents a significant difference (Tukey's HSD, p<0.05).

For the temperature data, results were less clear. Whilst the higher temperature (20 °C) removed greater quantities of higher alcohols (33% 1-propanol and 36% 3-methyl-1-butanol), lower temperatures removed greater amounts of ethyl acetate and acetaldehyde (Table 5.1). The data comparing operating pressures clearly shows advantages of volatile retention when operating at 20 bar without impacting operating duration. However, as results were not as clear for operating temperature, economic viability was taken into consideration and thus the higher temperature was selected for future trials, reducing energy output costs for cooling the dealcoholisation system.

Table 5.1: *Volatile aroma compound % change compared to start beer at 50% reduction in ethanol concentration for pressure and temperature trials (+ = % increase, - = % decrease). Different letters between compounds[ab] for either pressure or temperature trials represents a significant difference in % change (Tukey's HSD, p<0.05).*

	Pressure Trials		Temperature Trials	
Key Volatiles	20 bar	30 bar	10 °C	20 °C
Acetaldehyde	-0.9[b]	-61.4[a]	-95.5[a]	-13.6[b]
Ethyl Acetate	-19.4[b]	-54.7[a]	-47.2[a]	-38.8[b]
1-Propanol	-19.5	-24.9	-11.2[b]	-33.2[a]
Ethyl butyrate	+16.7[b]	-20.6[a]	+8.3	-22.7
Isobutanol	-8.3	-5.2	-7.2[b]	-29.4[a]

Isoamyl acetate	+5.3[b]	-17.8[a]	-1.8[b]	-28.5[a]
3-Methyl-1-butanol	-15.0[a]	-1.8[b]	-5.9[b]	-35.6[a]
Ethyl octanoate	-100.0	-100.0	-100.0	-100.0

Based on these pre-trials it was suggested that future research should use an RO90 membrane, operated at a pressure of 20 bar and temperature of 20 ºC to optimise ethanol reduction, whilst retaining key volatile compounds within the finished NAB product and reducing operating costs.

The next steps were to understand both the physicochemical and sensorial changes that occurred during dealcoholisation of different beer styles using RO techniques. Two very different beer styles were selected to understand how the beer matrix influences the dealcoholisation procedure. The need for different beer styles within a product range is apparent to appeal to the different wants and needs of consumers. Therefore, research is needed to understand whether brewers need to alter their dealcoholisation procedure for different beer styles.

This chapter was submitted as a paper to Food Chemistry in December 2020:

Ramsey, I., Yang, Q., Fisk, I., Ayed, C. and Ford. R. (2020) Assessing the sensory and physicochemical impact of reverse osmosis membrane technology to dealcoholize two different beer styles. *Journal of Membrane Science.*

Assessing the sensory and physicochemical impact of reverse osmosis membrane technology to dealcoholize two different beer styles

Imogen RAMSEY[ab], Qian YANG[a], Ian FISK[b], Charfedinne AYED[b], and Rebecca FORD[a]*

[a]Sensory Science Centre, Division of Food, Nutrition and Dietetics, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, United Kingdom

[b]Food Flavour Laboratory, Division of Food, Nutrition and Dietetics, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, United Kingdom

Key Words: Physicochemical, Sensory, Reverse Osmosis, Non-Alcoholic Beer, Dealcoholisation

Highlights:

- RO results in significant losses in volatile compounds and modified sensory profiles.
- Volatile losses appear to be related to compound structure, not compound size.
- RO efficiency varies between beer styles, with longer processing times for stouts.
- RO membranes are susceptible to fouling over time, affecting overall product quality.

Abstract:

Despite increased development of non-alcoholic beers (NABs), there is still a way to go in producing NABs that are sensorially similar to standard beer. This research aimed to understand physicochemical and sensorial changes between a standard beer and its dealcoholized counterpart using a pilot-scale dealcoholisation unit fitted with reverse osmosis (RO) membranes. Differences between product matrixes were explored by dealcoholizing two different beer styles, as well as evaluating the efficiency and consistency of RO membranes by performing replicate trials. Results showed clear differentiation between standard and dealcoholized beers, as key volatile compounds such as higher alcohols and esters were reduced along with ethanol. Compounds with increased levels of branching (including 3-methylbutyl acetate and 2-methylpropan-1-ol) were found to be retained to the highest level, in comparison to those with more linear structures. Sensory changes included loss of 'fruity/estery', 'alcoholic/solvent' and 'malty' aromas and flavours, 'sweetness' and 'body'. Key differences in membrane efficiency between beer styles were also found, resulting in longer processing times for the stout as trials progressed, suggesting membrane clogging. Identification of volatiles in the dealcoholised beers, which were not present in the starting matrix, suggests membrane fouling from previous products.

5.2 Introduction

Beer is the most consumed alcoholic beverage in the Americas and Europe (World Health Organisation, 2018), and the largest segment of the

drinks category in the UK, with 62% of the British population regular consumers (Mintel, 2018). Sales however, have fallen since 2012 by over 150 million litres, with factors suggesting this may be due to rising drinks prices and consumers limiting their alcohol consumption (Mintel, 2017b). Thus, a new revolution of health conscious consumers has appeared, leading an increase in sales of the non-alcoholic drinks sector. A third of consumers in the UK have reported they are limiting their alcohol consumption to improve their health, manage weight and reduce the risk of disease (Mintel, 2017a). This is driven by concerns due to the number of alcohol attributed deaths in the UK increasing year on year, which stood at 7,327 in 2016 and cost £21 billion per year in healthcare, crime and loss of productivity (Office For National Statistics, 2017). Consequently there has been increased interest in the development of non-alcoholic beers (NAB), with global manufacturers committing to responsible drinking targets by promising to increase their overall NAB range (ABInBev, 2018). A rise in the number of sales of NAB in European countries such as Spain and Germany has been observed, with output increasing almost 50% since 2014 (Euromonitor, 2017a) and total volume growth in the UK increasing by 29% between 2013 and 2018 (Euromonitor, 2019a). Nevertheless, there is still a way to go in producing a NAB which is sensorially similar to a standard beer in terms of flavour, taste and mouthfeel, with both consumer studies and market research reports stating that consumers find lower alcohol alternatives to be ‘bland’, ‘disappointing’ and ‘less tasty’ (Chrysochou, 2014, Mintel, 2015, Porretta and Donadini, 2008, Silva et al., 2016). 49% of consumers were also found to agree with the statement that lowering the alcohol content of a drink compromises the taste (Mintel, 2015).

Therefore, more research needs to be conducted to understand the key physicochemical and sensorial losses occurring during NAB production processes to tackle this issue.

NAB can be produced through numerous methods, which can be categorised into either biological or physical processing, however all of these methods will have some effect on the resulting sensory properties of the NAB. Biological processing includes arrested fermentation (by cooling, heating or limited yeast contact time), use of special yeasts (which produce little or no alcohol) and altered mashing processes (through reducing fermentable sugars in wort) (Branyik et al., 2012). Physical processing can be further categorised into thermal or membrane based processes. Thermal processes include rectification or thin film evaporation, using techniques such as spinning cone column or falling film evaporation (Andrés-Iglesias et al., 2015, Branyik et al., 2012, Catarino and Mendes, 2011b, Zufall and Wackerbauer, 2000b), whereas membrane processes can include dialysis, osmotic distillation, pervaporation, nanofiltration and reverse osmosis (RO) (Alcantara et al., 2016, Catarino and Mendes, 2011a, Catarino and Mendes, 2011b, Catarino et al., 2006, Catarino et al., 2007, De Francesco et al., 2015a, del Olmo et al., 2014, del Olmo et al., 2012, Labanda et al., 2009, Leskošek et al., 1995, Lopez et al., 2002, Pilipovik and Riverol, 2005, Zufall and Wackerbauer, 2000a). In a comprehensive study on NAB production, Branyik et al. (2012) found that all techniques produced significant losses in volatiles, however RO seemed to show the smallest change, with further encouraging results found from other researchers when dealcoholizing beer, wine and cider (Catarino et al., 2007, Gil et al., 2013,

Lopez et al., 2002, Pilipovik and Riverol, 2005, Alcantara et al., 2016, Catarino, 2010).

RO appears therefore to be one of the most promising techniques to produce a NAB. To summarise this technique, pressurised beer (20-80 bar) is passed through a semi-permeable membrane, meaning that the transmembrane pressure is above the osmotic pressure of the beer solution (Pilipovik and Riverol, 2005). Theoretically, the membrane is permeable to low molecular weight molecules such as water and ethanol, which are removed from the product into the permeate stream. The membrane is less permeable to larger molecules such as carbohydrates, colours and flavours, which can be fed back into the retentate beer tank (Müller et al., 2017). RO can be operated at low temperatures and pressures, reducing energy consumption with limited flavour losses (Catarino et al., 2007). As there are large water losses during processing, water needs to be added back in via diafiltration, which can be described as continuous (adding water back in during processing at different time points) or discontinuous (by diluting the product at the beginning or rediluting at the end to its original starting volume) (Branyik et al., 2012). The final product is then recarbonated, as CO_2 is lost during the process (Hodenberg, 1991). Membranes can be made from either cellulose acetate, polyamide or polyimide on polyester, polysulfone, or fibreglass support structures (Branyik et al., 2012) and are normally placed in geometric arrangement modules which can include planar, tubular or spiral-wound (Light et al., 1986). It has been found however, that the minimum achievable alcohol content is around 0.5% ABV, as it is not economically feasible to go below this (Catarino et al., 2007, Pilipovik and Riverol, 2005). Nevertheless, knowledge of flavour and sensorial differences in

the production of dealcoholised beer through RO is very limited, with little published data.

Previous studies conducted using RO have mainly focused on improving efficiency, by reporting on different operating parameters (pressure, temperature, membrane materials, and operating modes) with various alcoholic beverages (Catarino et al., 2006, Catarino et al., 2007, Falkenberg, 2014, Lopez et al., 2002, Pilipovik and Riverol, 2005). Higher pressures were found to result in increased permeate flux, higher rejection of ethanol and higher alcohols, and lower rejection of esters measured through gas chromatography (GC) (Catarino et al., 2007). Lower temperatures were also found to lower the permeate flux and increase rejection of aroma compounds (Catarino et al., 2007). Research conducted on different RO membrane materials with both cider (Lopez et al., 2002) and beer (Catarino et al., 2007) found that cellulose acetate membranes were the most promising, as they exhibited the highest permeate flux and lowest ethanol rejection. Similar results were found by Lopez et al. (2002), whilst also studying the use of different operating modes (continuous and discontinuous diafiltration). A key gap in research exists exploring the differences between replicate trials using membrane technology and the impact this has on subsequent trials. To date only one study, which was part of a MSc thesis, has reported this and found differences between subsequent runs using the same membranes in terms of ethanol reduction timings, discussing this to be due to soiling or fouling of the membranes (Falkenberg, 2014). Understanding membrane capabilities, as well as the potential changes in finished product quality, is important for breweries to understand, especially if membranes are prone to soiling or fouling. Changes

between trials can have a significant effect on the overall product quality for consumers, and therefore the importance in evaluating sensory and physicochemical properties between replicate trials needs to be addressed.

Few studies have evaluated the impact of dealcoholizing a standard strength beer using RO on the combined physicochemical and sensorial properties of the resulting NAB. Previous studies either only focused on key brewing parameters (such as colour, bitterness, pH, alcohol content, phenolic compounds and antioxidant activity) in a stout (Alcantara et al., 2016), or volatile profiles (using headspace solid phase microextraction gas chromatography (HS-SPME-GC-MS)) in a lager (Riu-Aumatell et al., 2014). Only one study combined HS-GC-MS techniques with sensory data, to assess the differences between lagers produced by different membrane filtration techniques (RO and NF), comparing them back to the original 5% beer (Falkenberg, 2014). Interestingly, RO gave the most similar results to the standard beer therefore showing that between the two methods, RO showed the most promise (Falkenberg, 2014). However, sensory analysis was not conducted using typical ISO standards, and should therefore be interpreted with caution. In addition, to the authors' knowledge no studies have directly compared the impact of dealcoholisation via RO on different beer styles. It is hypothesised here, that a difference in the starting matrix through the use of different raw materials may have an effect on the membrane efficiency, resulting in changes to the physicochemical and sensory properties of the resulting NABs. This is valuable information to brewers, as the same RO equipment could potentially be used to develop a range of NAB styles to satisfy a range of consumer needs.

Therefore, in the present study, the impact of RO on the physicochemical and sensory properties of different beer styles was assessed to understand the efficacy of this method for producing lower alcohol versions of standard beers. The objectives of this study were therefore to explore the use of dealcoholisation using RO membranes on i) the key physicochemical and sensorial properties of two different beer styles compared to their standard strength equivalents; ii) the influence of compound characteristics (molecular weight, Log*P* and structure) on their removal; iii) matrix-membrane interactions; iv) membrane efficiency by performing replicate trials.

5.3 Experimental

5.3.1 Beer Samples

For the purpose of this study, 300 L of both a lager and a stout were purchased from a local brewery. These were delivered as 6 x 50 L kegs, which were all from the same overall batch of beer (East Sussex, UK). The lager purchased was a 5.1% ABV Pilsner with the following ingredients: lager malt, cara pils malt, Mittlefruh leaf hops and Saaz leaf hops, SafLager W-34/70 yeast. The stout was a 4.3% ABV oatmeal stout with the following ingredients: pale ale, chocolate, wheat, light crystal and Carafa Spieziel III malts, flaked oats, roasted barley, Fuggles hops and Saale US-05 yeast.

5.3.2 Dealcoholisation

Dealcoholisation tests were conducted using a pilot-scale LabStak M20-0.72 unit (Alfa Laval, Lund, Sweden) fitted with an RO90 spiral wound membrane made up of a thin film composite polyamide membrane with

polyester support material, measuring 1.9 m² (Alfa Laval, Lund, Sweden). For all trials, modifications were made to ensure that the M20-0.72 unit was operated in a 'closed' environment, pressurised with CO_2 and suitable for use within a commercial setting. For each replicate trial performed, 50 L beer was introduced from the purchased commercial keg into the sample tank, which had a maximum capacity of 160 L. The unit was then turned on, allowing the pump to process beer from the sample tank through the membrane. Ethanol and water were removed through the permeate tube and dealcoholized beer was processed back into the sample tank to be dealcoholized again. This process was performed on a continuous loop until the beer reached its desired ethanol concentration. At regular time points deaerated brewing liquor, pressurised with CO_2 to avoid oxygen problems, was added back into the sample tank following continuous diafiltration. Previous trials confirmed the selection of membrane type to be used (RO90), operating temperature (20 °C) and trans-membrane pressure (20 bar) by calculating ethanol reduction efficiency, least volatile reduction and economic viability. Temperature was controlled before entering the membrane module by the use of a temperature controlled valve, with cooling water used as a cooling medium for the sample and deaerated brewing liquor tanks. Pressure was controlled using the RO pressure dial located on the unit. A basic diagram of the set-up is shown in figure 5.3. Before starting dealcoholisation, 3 x 50 L kegs of each original beer style, each keg was labelled as replicates 5A, 5B and 5C and then beer was transferred into 275 mL bottles. All dealcoholisation trials were performed in triplicate to understand the efficiency of the membrane. Once dealcoholized, the beer was

transferred into 275 mL bottles and labelled according to the replicate trial: e.g. 0A, 0B and 0C for separate dealcoholised trials.

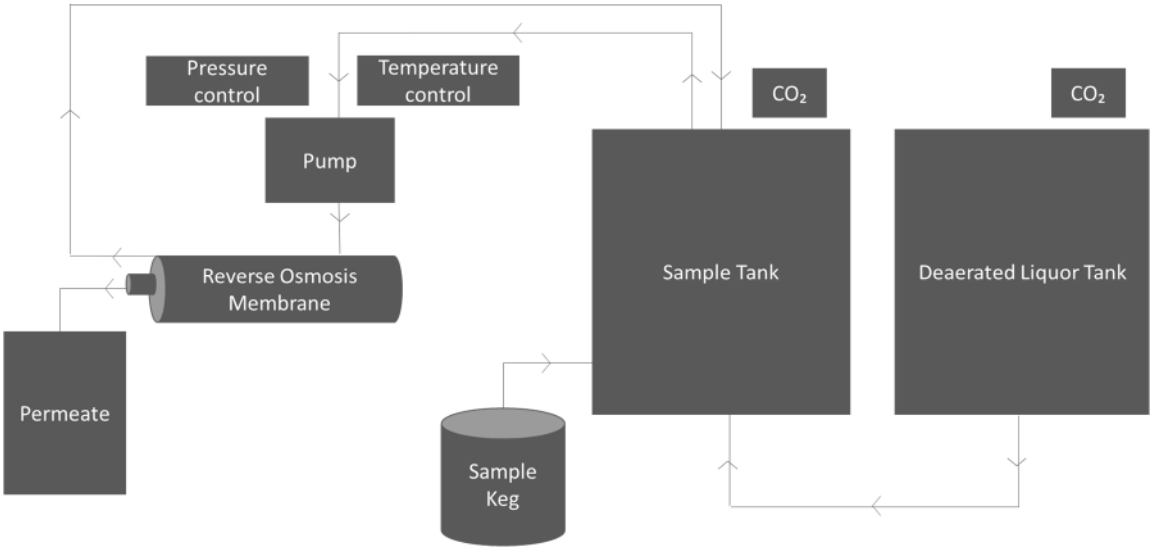


Figure 5.3: *Reverse osmosis dealcoholisation set up in a closed system for trials*

5.3.2.1 Membrane Cleaning

Cleaning followed the manufacturers' membrane cleaning guidelines, by flowing mains water through the system for 20 min. Subsequently, a 0.1% NaOH solution at 30-40 °C was circulated for 20 min and then rinsed with mains water again for 20 min. This procedure was completed after every trial.

5.3.3 Physicochemical Analysis

Instrumental analyses were conducted to investigate the differences in beer styles and their key chemical characteristics. Ethanol content was measured using an Anton Paar Alcolyzer and DMA4500 (Graz, Austria). Sample pH was determined using a Metler Toledo FiveGo pH meter (Colombus, Ohio, USA) after calibration with pH 4.0 and 7.0 standards. Bitterness units (BU) were determined using the international method by the

American Society of Brewing Chemists (ASBC) (Beer-23A) (ASBC Method of Analysis, 2018). Beer (5 mL) was transferred into a 50 mL centrifuge tube and acidified with 3 M HCl (0.5 mL). Isooctane (10 mL) was added and the mixture was shaken by hand three times, placed on a mechanical shaker for 15 min, centrifuged at 400 xg for 5 min, and then again for another 5 min to aid phase separation. The clear isooctane layer was then transferred into a cuvette and absorbance was measured at 275 nm with a spectrophotometer against a blank of isooctane. The recorded absorbance was multiplied by 50 to give BU values in mg/L. Total polyphenol (TP) content was also determined using the international method by the ASBC (Beer-35) (ASBC Method of Analysis, 2015). Beer (10 mL) was mixed with a preparation of carboxymethylcellulose (CMC, 1%) and ethylenediamine tetraacetic acid (EDTA, 0.2%) (8 mL) in a 25 mL volumetric flask. Ferric acid (0.5 mL) and ammonia (0.5 mL) were then added, with mixing after each addition. The solution was then made up to mark with RO water, left to stand at room temperature for 10 min, and absorbance was measured at 600nm with a spectrophotometer against a blank of the beer sample (mixed with CMC/EDTA and ammonia). The recorded absorbance was multiplied by 820 to give total polyphenol values in mg/L.

Headspace Gas Chromatography-Flame Ionization Detector (HS-GC-FID) lower boiling point beer volatile analysis was determined using the method proposed by Analytica-European Brewing Convention (EBC) (9.39) (Analytica-EBC, 2018). Beer samples (10 mL) were transferred into glass vials with 3.5 g sodium chloride and 50 µL 1-butanol (internal standard). Volatiles were analysed with a Scion 456-Gas Chromatograph (Scion Instruments, West Lothian, UK). Samples (500 µL) were incubated at 60 °C for 20 min with

shaking, and then were injected in splitless mode using a PAL Combi-XT autosampler (PAL System, Zwingen, Switzerland) onto a Zebron ZBWax column (60m x 0.25 ID; Phenomenex Inc, Cheshire, UK). Column temperature was held initially at 85 ˚C for 10 min, increased by 25 ˚C/min to 110 ˚C, before finally being increased by 8 ˚C/min to 200 ˚C. Total run time was 36.25 min. The GC carrier gas was helium, at a constant pressure of 15 psi. Full scan mode was used to detect volatile compounds (mass range from m/z 35 to 200). Volatile compounds were identified by their m/z, and quantified with the use of pure and internal standards. The following aroma compounds were purchased from Sigma-Aldrich (UK) for standard identification: acetaldehyde (≥99.5%), ethyl acetate (≥99.5%), 2-methylpropyl ethanonate (≥97%), propan-1-ol (≥99%), 3-methylbutyl acetate (≥97%), 3-methyl-1-butanol (≥99%), ethyl octanoate (≥98%) and ethyl decanoate (≥98%). Other compounds were purchased from Thermo Fisher Scientific (UK): 1-butanol (≥99.5%), ethyl butanoate (≥99%), 2-methylpropan-1-ol (≥99%) and ethyl hexanoate (≥99%).

To detect other relevant volatile compounds not found through HS-GC-FID analysis, Solid Phase Microextraction (SPME) and liquid extraction (LE) were used. For SPME analysis, beer samples (5 mL) were transferred into glass vials and 100 µL 3-heptanone (internal standard) was added and analysed using a modified published method by Yang et al. (2016). Modifications to the method included incubation of samples at 40 °C for 2 min with shaking, with volatile aroma compounds extracted for 10 min and desorped for 1 min. Column temperature was held initially at 40 °C for 2 min, increased by 8 °C/min to 240 °C and held for 1 min. Total run time was 38 min. For LE analysis, beer samples (20 mL) were transferred into a 50 mL conical-based

glass tube with 2 mL dichloromethane (DCM) and 100 µL 3-heptanone (internal standard) using a modified published method by Holmes et al. (2014). The tube was sealed with a PTFE lined cap and placed on a roller bed at room temperature (150 rpm for 1 h). After extraction, samples were centrifuged at 1000 rpm for 2 min and then the DCM layer was transferred into a glass vial ready for analysis. DCM extracts were analysed with a Scion 456-Gas Chromatograph (Scion Instruments, West Lothian, UK). Samples (1 µl) were injected in splitless mode using a PAL Combi-XT autosampler (PAL System, Zwingen, Switzerland) onto a Zebron ZBWax column (60 m x 0.25 ID; Phenomenex Inc, Cheshire, UK). The GC carrier gas was helium, at a constant pressure of 18 psi. Column temperature was held initially at 40 ˚C, and then increased by 6 ˚C/min to 225 ˚C. Full scan mode was used to detect volatile compounds for both SPME and LE methods (mass range from m/z 35 to 200). Volatiles were identified by their m/z and comparison of each mass spectrum with either the spectra from authentic compounds or with spectra in reference libraries (NIST/EPA/NIH Mass Spectral Library, version 2.0, Faircom Corporation, U.S). The quantification of volatiles was expressed by the peak area ratio (PAR), which was calculated by the GC peak area for the compound divided by the peak area of the internal standard.

5.3.4 Sensory Analysis

The sensory attributes of the lager and stout samples were evaluated by trained beer panellists (n=12) from the Campden BRI beer panel using a modified quantitative descriptive analysis (QDA) approach (Stone and Sidel, 2004). Assessors had a minimum of 100 h experience in generic descriptive analysis of beer samples. Panel monitoring and training occurred through

participation in LGC Standards Proficiency Testing (Teddington, Middlesex, UK) Brewing Analytes-Chemistry (BAPS-CHEM) Level 5 Sensory. Panellists also received monthly refresher training sessions with attributes, definitions and reference standards (data not shown, Appendix Table 6), to assess their ability to describe, discriminate and replicate. All attributes were evaluated using a continuous unstructured line scale, with marks converted to a score of ten for data analysis purposes.

Final sample evaluation was carried out at the Campden BRI sensory facility (Nutfield, Surrey, UK) conforming to ISO standards (ISO 8589: 2007) and included three sessions for each beer style, allowing for triplicate evaluation of each sample by each panellist. Beer samples (50 mL), labelled with three-digit codes, were served at 12±2 ° C in lidded black glasses under red light in a balanced, blocked and randomised presentation order. A maximum of six samples were evaluated per two-hour session, with a 10 min break after every two samples, to ensure no carryover or fatigue effects. Panellists were instructed to assess each sample for aroma, taste and mouthfeel attributes using Compusense cloud™ (Guelph, Canada) and were told to expectorate the sample after evaluating. The order of attributes was agreed with panellists before final evaluation took place, starting with the attribute that was perceived first and ending with the last. Unsalted crackers (Tesco, UK) and filtered water (Brita filter jug) were provided for palate cleansing.

5.3.5 Statistical Analysis

Analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) post hoc test were conducted at $p<0.05$ for instrumental analysis. To identify the difference between the original and

dealcoholized beer for each trial replicate, the % decrease was calculated. All analyses were conducted in duplicate across three sample bottles from the same batch, and a mean calculated. Compounds detected were sorted into size (through number of carbon atoms and molecular weight) to understand the impact on their removal by the membrane. As a further means of assessing the influence of compound characteristics (molecular weight, Log*P*, structure), molecular operating environment (MOE) (2002.03, Chemical Computing Group, Montreal, Canada) molecular descriptors were used. Partial least squares regression (PLS-R) analysis was conducted, with the relative peak areas of the volatile compounds obtained from GC-MS analysis for each beer style as the dependent variable (X-matrix). This was calculated as a ratio of the peak area of the 5% beer compared to the 0% beer. Molecular descriptors acted as independent variables (Y-matrix) to model the relation between these variables. All mean-centred relative peak areas were initially subjected to PLS-R for dimensionality reduction, and the independent variables (molecular descriptors) for which Variable Importance in Projection (VIP) values were less than 1 were excluded from further analysis, as they can be considered to not contain enough information to explain the variance of data. The remaining descriptors were subjected to a second PLS-R, where the total variance of the dataset was cumulatively explained by a limitless number of variables. The scores of the first three molecular descriptors were extracted and used as key variables in the model parameters as they provided the best linear regression between the model equation and the raw data (R^2>0.5, Q^2>0.5).

A two factor ANOVA (sample, panellist) with interaction and Tukey's HSD post hoc test was performed on sensory results.

In order to explore the relationships between physicochemical properties and sensory data for each beer style, a PCA was conducted. Both datasets used averaged scores across samples and only included sensory attributes and compounds which significantly discriminated amongst the samples, assessed by ANOVA. Data analyses were performed using XLSTAT (v19.01, Addinsoft, New York, USA).

5.4 Results

5.4.1 Trials

Details of replicate trials for both lager and stout are shown in Table 5.2.

5.4.1.1 Lager

The dealcoholized lager replicate trials (0A, 0B, 0C) all showed similar starting volumes and final ethanol concentrations. Interestingly, the permeate flowrate reduced with trials, from 420 mL/min in the first trial to 350 mL/min in the third trial. The run time also showed some differences, with trials 0A and 0C showing shorter times compared to trial 0B.

5.4.1.2 Stout

Two of the dealcoholized stout replicate trials (0A, 0B) also showed similar final ethanol concentrations. Unfortunately, however the third replicate (0C) had a smaller starting volume, due to loss of original beer when transferring from keg to sample tank, resulting in a lower final ethanol concentration and shorter run time. Interestingly the permeate flowrate for the stouts increased during trials, yet it was still a lot lower than for the lager. This

meant that the duration of dealcoholisation run times were significantly higher for the stout averaging around 10 hours compared to 7 hours for the lager.

Table 5.2: *Starting product volume, initial and final ethanol concentrations, permeate flowrate and run time of three replicate trials for lager and stout style beers using pilot scale LabStak M20-0.72 unit*

Beer Style	Lager			Stout		
Replicate	0A	0B	0C	0A	0B	0C
Starting Product Volume (L)	50.5	51.2	44.2	51.6	52.4	18.0
Initial Ethanol Concentration (ABV)	5.09	5.09	5.10	4.31	4.29	4.30
Final Ethanol Concentration (ABV)	0.45	0.47	0.40	0.35	0.35	0.08
Permeate Flowrate (mL/min)	420	385	350	230	250	272
Run time	6 hours 43mins	7 hours 30mins	6 hours 30mins	10 hours 35mins	9 hours 50mins	3 hours 03mins

5.4.2 Physicochemical Results

Physicochemical analysis for each trial are presented for the lager (Table 5.4a) and the stout (Table 5.4b). Results for ethanol concentration showed a significant reduction after dealcoholisation for all replicates, with around 91% for all lager trials, 92% for stout trials A and B and 98% for stout trial C - due to a smaller starting volume for stout trial C. The results from these trials therefore confirmed that RO is a suitable technique for removing ethanol from beer. Bitterness and total polyphenol content were also shown to decrease for all trials, with a decrease in pH shown for the lager trials, but an increase shown for the stout trials. HS-GC-FID analysis allowed the identification and quantification of the most abundant compounds, showing that the concentration

of all compounds for both lager and stout in each of the dealcoholisation trials were significantly different ($p<0.05$) from the starting concentration. Interestingly, a difference amongst replicate trials for each beer style was also shown. For both the lager and the stout, increased removal of higher alcohols (propan-1-ol, 2-methylpropan-1-ol and 3-methyl-1-butanol) was shown for trial 0C in comparison to trials 0A and 0B. Esters (ethyl acetate, ethyl butanoate and 3-methylbutyl acetate) also followed a similar trend for the stout. Finally, a small increase was shown for 2-methylpropyl ethanonate, with reasons for this discussed in latter sections.

Further analysis was performed using SPME-GC-MS and LE-GC-MS to understand reductions of compounds not found through HS-GC-FID (shown in Table 5.5). Most compounds were found to significantly decrease after dealcoholisation for both lager and stout trials, showing that it is not just ethanol that is removed when using RO membranes. However, differences between replicate trials for each beer were found suggesting a lack of consistency. Some compounds were also interestingly found to increase after dealcoholisation.

PLS-R was used as an attempt to model the relationship between volatile compounds, molecular descriptors and their removal from the beer. Of the 105 molecular descriptors explored, three main molecular descriptors were found, which can be used to explain the pathway of certain molecules through the membrane. Interestingly, the same descriptors were found for both beer styles, which included one surface area, volume and shape descriptor (*pmiZ)* and two subdivided surface areas descriptors (*SlogP_VSA3*, *SMR_VSA7)* (more information provided in Table 5.3).

Table 5.3: *Main molecular descriptors discovered by PLS-R analysis and their definitions from MOE (2002.03, Chemical Computing Group, Montreal, Canada)*

Molecular Descriptor	Definition
pmiZ	Spatial external 3D descriptor based on the z component of principal moment of the inertia.
SlogP_VSA3	Represents the Van der Vaals surface area of the atoms contributing to the logP (o/w) of the molecule in the range (0, 0.1).
SMR_VSA7	Sum of νi, such that Ri > 0.56. This is the subdivided surface area based on an approximate accessible van der Waals surface area (in $Å^2$) calculation for each atom, ν_i, along with some other atomic property, p_i.

5.4.3 Sensory Results

The mean attribute scores and results from ANOVA and Tukey's HSD for the twenty-four aroma, flavour, taste and mouthfeel attributes for the NAB and full strength lagers using QDA with the trained panel were calculated.

5.4.3.1 Lager

ANOVA revealed differences for 'fruity/estery aroma', 'alcoholic/solvent aroma', 'fruity/citrus aroma', 'malty aroma', 'fruity/estery flavour', 'alcoholic/solvent flavour', 'fruity/citrus flavour', 'malty flavour', 'other sulfur flavour', 'sweet' and 'sour' tastes, 'linger' aftertaste and 'body' attributes ($p<0.0001$). A spider plot (Figure 5.4a), shows average ratings and significant sensory attribute terms for each trial of both 5% (original) and 0%

(dealcoholised) ABV samples. Samples 5A, 5B and 5C were found to be significantly higher ($p<0.0001$) for the attributes 'fruity/estery aroma', 'alcoholic/solvent aroma' and 'malty aroma', 'fruity/estery flavour', 'alcoholic/solvent flavour' and 'malty flavour', 'sweet' and 'body' compared to the dealcoholised samples (0A, 0B, 0C). However, for samples 0A, 0B and 0C 'sour' was significantly higher ($p<0.0001$). The attributes 'fruity/citrus aroma' and 'fruity/citrus flavour' and 'linger' showed no significant difference between the 5% and 0% ABV samples, however differences between dealcoholized samples were discovered, with trial 0C having significantly lower amounts of 'fruity/citrus aroma' and 'fruity/citrus flavour' compared to trials 0A and 0B.

5.4.3.2 Stout

For the stout, ANOVA revealed significant differences for 'alcoholic/solvent aroma', 'fruity/citrus aroma', 'hop aroma', 'cereal aroma', 'malty aroma' and 'burnt aroma', 'alcoholic/solvent flavour', 'fruity/citrus flavour', 'hop flavour', 'cereal flavour', 'malty flavour', 'burnt flavour', 'caramel flavour', 'other sulfur flavour' and 'other flavours', 'sweet' taste, 'linger' aftertaste and 'body' attributes. Figure 5.4b shows that samples 5A, 5B and 5C were significantly higher ($p<0.0001$) for the attributes 'alcoholic/solvent aroma', 'burnt aroma', 'alcoholic/solvent flavour', 'fruity/estery flavour', 'fruity/citrus flavour', 'hop flavour', 'malty flavour', 'caramel flavour', 'sweet' and 'body' than 0A, 0B and 0C. However, for samples 0A, 0B and 0C 'fruity/citrus aroma' was significantly higher ($p<0.0001$). Differences between dealcoholized samples were also shown, with

higher levels of ‘cereal aroma’, ‘malty aroma’ and ‘burnt aroma’ and decreased ‘linger’ in trial 0C compared to 0A and 0B.

Table 5.4a: *Physicochemical results (ABV, pH, Bitterness Units, Total Polyphenols and Lower Boiling Point Volatiles for lager trials A, B and C). % change was calculated for each trial replicate as a percentage left from the original beer to the dealcoholized beer. Different letters within a row[abc] represent a significant difference among samples in terms of volatile concentrations (Tukey's HSD, p<0.05).*

Measurements		Trial A			Trial B			Trial C		
		Original Beer (5A)	Dealcoholised Beer (0A)	% Change	Original Beer (5B)	Dealcoholised Beer (0B)	% Change	Original Beer (5C)	Dealcoholised Beer (0C)	% Change
Ethanol Concentration (ABV)		5.09[a]	0.45[b]	*-91.1*	5.09[a]	0.40[c]	*-92.1*	5.10[a]	0.47[b]	*-90.8*
pH		4.50[a]	4.38[b]	*-2.6*	4.50[a]	4.36[b]	*-3.1*	4.51[a]	4.37[b]	*-3.2*
Bitterness Units		16.32[a]	11.36[c]	*-30.4*	14.93[b]	11.49[c]	*-23.0*	16.16[ab]	10.42[c]	*-35.5*
Total Polyphenols (mg/L)		282.08[a]	239.80[bc]	*-15.0*	262.58[ab]	224.68[c]	*-14.4*	272.42[a]	216.85[c]	*-20.4*
Volatile Compounds (mg/L)	Acetaldehyde	11.90[a]	3.42[c]	*-71.3*	12.24[a]	1.96[c]	*-84.0*	12.09[a]	6.78[b]	*-43.9*
	Ethyl Acetate	45.73[a]	3.83[b]	*-91.6*	39.88[a]	3.46[b]	*-91.3*	46.58[a]	4.75[b]	*-89.8*
	2-Methylpropyl Ethanoate	0.00[b]	0.02[b]	*+100*	0.00[b]	0.01[ab]	*+100*	0.00[b]	0.01[b]	*+100*
	Propan-1-ol	25.35[a]	9.77[b]	*-61.4*	22.74[a]	8.79[b]	*-61.4*	23.67[a]	4.40[c]	*-81.4*
	Ethyl Butanoate	0.10[a]	0.02[b]	*-79.3*	0.08[a]	0.02[b]	*-75.5*	0.09[a]	0.01[b]	*-86.8*
	2-Methylpropan-1-ol	23.59[a]	13.80[b]	*-41.5*	21.21[a]	12.37[b]	*-41.7*	22.38[a]	4.96[c]	*-77.8*
	3-Methylbutyl Acetate	3.60[a]	0.54[b]	*-85.0*	2.80[a]	0.49[b]	*-82.5*	3.33[a]	0.66[b]	*-80.2*
	3-Methyl-1-Butanol	146.92[a]	45.65[c]	*-68.9*	129.46[ab]	41.87[c]	*-67.7*	136.78[b]	37.20[c]	*-72.8*
	Ethyl Hexanoate	0.36[a]	0.03[b]	*-92.2*	0.26[a]	0.02[b]	*-92.2*	0.29[a]	0.00[b]	*-100*
	Ethyl Octanoate	0.34[a]	0.01[c]	*-96.1*	0.13[b]	0.00[c]	*-100*	0.14[b]	0.01[c]	*-90.4*

Table 5.4b: *Physicochemical results (ABV, pH, Bitterness Units, Total Polyphenols and Lower Boiling Point Volatiles for stout trials A, B and C). % change was calculated for each trial replicate as a percentage left from the original beer to the dealcoholized beer. Different letters within a row[abc] represent a significant difference among samples in terms of volatile concentrations (Tukey's HSD, p<0.05).*

Measurements		A			B			C		
		Original Beer (5A)	Dealcoholised Beer (0A)	% Change	Original Beer (5B)	Dealcoholised Beer (0B)	% Change	Original Beer (5C)	Dealcoholised Beer (0C)	% Change
Ethanol Concentration (ABV)		4.31[a]	0.35[d]	*-91.9*	4.29[c]	0.35[d]	*-91.8*	4.30[b]	0.08[e]	*-98.1*
pH		4.09[b]	4.17[a]	*+2.0*	4.08[b]	4.15[a]	*+1.7*	4.09[b]	4.16[a]	*+1.7*
Bitterness Units		23.71[a]	17.03[b]	*-28.2*	24.13[a]	15.58[b]	*-35.4*	23.93[a]	15.12[b]	*-36.8*
Total Polyphenols (mg/L)		381.66[a]	258.94[bc]	*-32.2*	392.87[a]	243.63[c]	*-38.0*	340.94[ab]	237.16[c]	*-30.4*
Volatile Compounds (mg/L)	Acetaldehyde	3.11[bc]	3.42[b]	*+9.7*	3.95[ab]	1.96[c]	*-50.3*	3.52[ab]	4.64[a]	*+31.8*
	Ethyl Acetate	19.32[a]	3.83[b]	*-80.2*	19.93[a]	3.46[b]	*-82.6*	20.48[a]	1.11[c]	*-94.6*
	2-Methylpropyl Ethanoate	0.00[b]	0.01[a]	*+100*	0.01[ab]	0.01[ab]	*0*	0.00[b]	0.00[b]	*0*
	Propan-1-ol	62.84[a]	9.77[b]	*-84.4*	61.95[a]	8.79[b]	*-85.8*	66.30[a]	2.13[c]	*-96.8*
	Ethyl Butanoate	0.07[a]	0.02[b]	*-72.7*	0.08[a]	0.02[b]	*-73.3*	0.07[a]	0.00[c]	*-100*
	2-Methylpropan-1-ol	50.87[a]	13.80[b]	*-72.9*	50.30[a]	12.37[b]	*-75.4*	50.97[a]	3.65[c]	*-92.8*
	3-Methylbutyl Acetate	1.54[a]	0.54[b]	*-65.1*	1.58[a]	0.49[b]	*-68.9*	1.50[a]	0.19[c]	*-87.5*
	3-Methyl-1-Butanol	146.80[a]	45.65[b]	*-68.9*	141.30[a]	41.87[b]	*-70.4*	143.80[a]	16.19[c]	*-88.7*
	Ethyl Hexanoate	0.26[a]	0.03[b]	*-89.2*	0.26[a]	0.02[b]	*-92.3*	0.23[a]	0.00[b]	*-100*
	Ethyl Octanoate	0.21[b]	0.01[c]	*-93.5*	0.26[a]	0.00[c]	*-100*	0.20[b]	0.00[c]	*-99.2*

Table 5.5: *LogP values and molecular weight of compounds detected by SPME-GC-MS and LE-GC-MS, with % change for each beer style replicate dealcoholisation trial calculated from original beer peak area minus dealcoholized beer peak area. LogP values found through EPI Suite™ (4.11, U.S Environmental Protection Agency, Washington, USA). Identification method included (AS= authentic standard, N= mass spectrum compared to NIST database).*

	Carbon Atoms	Compound Name	LogP	Molecular weight	Identification Method	Lager % Change			Stout % Change		
						A	B	C	A	B	C
Esters	C4	Ethyl Acetate (1)	0.86	88.11	AS	-92	-91	-90	-80	-83	-95
		Ethyl Propanoate (2)	1.36	102.13	AS	-60	-62	-63	-81	-56	-51
		Propyl Acetate (3)	1.36	102.13	N	-71	-70	-75	-66	-66	-100
	C5	Ethyl 2-hydroxypropanoate (4)	-0.18	118.13	N	-100	-100	-100	-100	-100	-100
		Ethyl Butanoate (5)	1.85	116.16	AS	-79	-76	-87	-73	-73	-100
	C6	2-Methylproyl Ethanoate (6)	1.77	116.16	AS	+100	+100	+100	+100	0	0
		Methyl Hexanoate (7)	2.34	130.18	AS	-60	-62	-64	-65	-67	-100
		Ethyl Pentanoate (8)	2.34	130.18	AS	-100	-100	-100	-100	-100	-100
		Ethyl 3-methylbutanoate (9)	2.26	130.19	AS	-82	-81	-78	-36	-25	-100
		Pentyl Acetate (10)	2.34	130.19	N	-90	-92	-89	-100	-100	-100
	C7	3-Methylbutyl Acetate (11)	2.26	130.19	AS	-85	-83	-80	-65	-69	-88
		Ethyl Hexanoate (12)	2.83	144.21	AS	-92	-92	-100	-89	-92	-100
		Hexyl Acetate (13)	2.83	144.214	AS	-84	-85	-84	-77	-81	-92
	C8	2-Methylpropyl 2-Methylpropanoate (14)	2.51	144.21	N	-24	-42	-35	-16	-9	-75
		Methyl Octanoate (15)	3.32	158.24	AS	+83	+92	+83	+56	+34	+12
		Ethyl Heptanoate (16)	3.32	158.24	AS	-100	-100	-100	-100	-100	-100
		Heptyl Acetate (17)	3.32	158.24	AS	-100	-100	-100	-100	-100	-100
	C9	2-Methylbutyl 2-Methylpropanoate (18)	3.66	158.24	AS	-41	-51	-59	-44	-48	-78
		Ethyl Octanoate (19)	3.81	172.268	AS	-96	-100	-90	-94	-100	-99
	C10	2-Phenylethyl Acetate (20)	2.57	164.2	AS	-80	-78	-79	-100	-100	-100
	C11	Methyl Decanoate (21)	4.3	186.29	N	+76	+88	+72	N/A	N/A	N/A
		Ethyl Decanoate (22)	4.79	200.322	AS	-96	-100	-100	-100	-100	-100
	C12	a-Terpineol acetate (23)	4.34	196.29	AS	+85	+82	+79	+100	+100	+100
Alcohols	C3	Propan-1-ol (24)	0.35	60.09	AS	-61	-61	-81	-84	-86	-97
	C4	2-Methylpropan-1ol (25)	0.77	74.122	AS	-42	-42	-78	-75	-75	-93
		2-Furanmethanol (26)	0.45	112.13	AS	-96	-100	-100	-100	-100	-100
	C5	3-Methyl-1-Butanol (27)	1.26	88.148	AS	-69	-68	-73	-69	-70	-89
	C6	Hexan-1-ol (28)	1.75	102.162	AS	-77	-73	-77	-75	-78	-99
		5-Methylfurfuryl alcohol (29)	1.38	112.13	AS	N/A	N/A	N/A	-100	-100	-100

	C7	Heptan-1-ol (30)	2.24	116.88	AS	-100	-100	-100	-100	-100	-100
		Octan-1-ol (31)	2.73	130.23	AS	-100	-100	-100	-100	-100	-100
		Oct-1-en-3-ol (32)	2.60	128.21	AS	-100	-100	-100	-100	-100	-100
	C8	2-Phenylethan-1-ol (33)	1.57	122.16	AS	-79	-79	-82	-79	-79	-94
	C9	Nonan-2-ol (34)	3.22	144.25	AS	-70	-100	-91	-100	-100	-100
		3,7-Dimethyl-1,6-octadien-3-ol (35)	3.38	154.25	AS	+88	+87	+77	+90	+87	+70
		Terpinen-4-ol (36)	3.33	154.25	AS	+100	+100	+100	+100	+100	+100
		2-(4-methylcyclohex-3-en-1-yl)propan-2-ol (37)	3.33	154.25	AS	+100	+100	+100	+100	+100	+100
	C10	1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane (38)	3.00	154	AS	+100	+100	+100	+100	+100	+100
Carboxylic Acids	C2	Acetic Acid (39)	0.09	60.05	AS	-94	-90	-93	-100	-100	-100
		Butanoic Acid (40)	1.00	88.11	AS	-100	-100	-100	-100	-100	-100
		2-Methylpropanoic Acid (41)	0.94	88.11	AS	-100	-100	-100	-86	-94	-100
	C4	2-Methylbutanoic Acid (43)	1.49	102.13	AS	-100	-100	-100	-100	-100	-100
		3-Methylbutanoic Acid (44)	0.11	102.13	AS	+85	+94	+94	+85	+94	+94
	C6	Hexanoic Acid (45)	2.05	116.16	AS	-100	-100	-100	-100	-100	-100
Aldehydes	C2	Acetaldehyde (46)	0.36	44.05	AS	-71	-84	-44	+10	-50	+32
	C5	Furan-2-carbaldehyde (Furfural) (47)	0.83	96.08	AS	-63	-47	-41	-28	-46	-63
	C6	1-Methylpyrrole-2-carbaldehyde (48)	1.43	109.13		N/A	N/A	N/A	-100	-100	-100
	C8	2-Phenylacetaldehyde (49)	1.54	120.15	AS	-100	-100	-100	N/A	N/A	N/A
Ketones	C4	Butane-2,3-dione (50)	-1.34	86.09	AS	-78	-77	-69	-74	-100	-100
	C7	1-(furan-2-yl)propan-1-one (51)	0.80	124.05		N/A	N/A	N/A	-100	-100	-100
Lactones	C6	2-Oxepanone (52)	0.68	114.14		-100	-100	-100	N/A	N/A	N/A
		3-Hydroxy-4,5-dimethylfuran-2(5*H*)-one (53)	-0.44	128.13		-42	-43	-45	-45	-67	-100
Hydr	C6	Benzene (54)	1.99	78.11	N	+49	+43	+46	47	42	52
Am	C5	Valine (55)	-2.08	117.151		-46	-38	-42	-55	-41	-78
Terpenes		1-Methyl-4-(propan-2-yl)benzene (56)	4.00	134.21	N	+100	+100	+100	+100	+100	+100
		(1*R*,4*S*,6*S*)-4,7,7-trimethylbicyclo[4.1.0]hept-2-ene (57)	4.48	136.24	N	+100	+100	+100	+100	+100	+100
	C10	1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (58)	3.04	152.23	N	+100	+100	+100	+82	+100	+100
Diol	C3	Propane-1,3-diol (59)	-1.09	76.09	AS	-81	-100	-100	-78	-85	-100
Alkyl Sulphid	C4	3-Methylsulfanylpropan-1-ol (60)	0.44	106.19	AS	-100	-100	-100	-100	-100	-100
		Thiolan-3-one (61)	-1.78	102.01	N	N/A	N/A	N/A	-100	-100	-100
Diaz	C4	Pyrimidine (62)	-0.06	80.88	AS	N/A	N/A	N/A	-100	-100	-100
Pyrazine	C5	2-Methylpyrazine (63)	0.49	94.11	AS	N/A	N/A	N/A	-84	-95	-100
		2-Ethylpyrazine (64)	0.98	94.11	N	N/A	N/A	N/A	-100	-100	-100
		2,3-Dimethylpyrazine (65)	1.03	108.14	AS	N/A	N/A	N/A	-100	-100	-100
		2,5-Dimethylpyrazine (66)	1.03	108.14	AS	N/A	N/A	N/A	-100	-100	-100
	C6	2,6-Dimethylpyrazine (67)	1.03	108.14	N	N/A	N/A	N/A	-100	-100	-100

		2-Ethyl-3-methylpyrazine (68)	1.53	122.17	AS	N/A	N/A	N/A	-40	-43	-48
	C7	2-Ethyl-6-methylpyrazine (69)	1.53	122.17	N	N/A	N/A	N/A	-70	-83	-96
		3-Ethyl-2,5-dimethylpyrazine (70)	2.07	136.19	AS	N/A	N/A	N/A	-51	-100	-100
	C8	5-Methyl-6,7-dihydro-5H-cyclopentapyrazine (71)	1.83	134.18	N	N/A	N/A	N/A	-64	-100	-100
Furan	C6	1-(furan-2-yl)ethanone (72)	0.80	110.11	N	N/A	N/A	N/A	-85	-89	-100
	C9	2-Pentylfuran (73)	3.87	138.21	AS	N/A	N/A	N/A	-100	-100	-100


A
Fruity/Estery - A***
Alcoholic/Solvent - A***
Fruity/citrus - A*
Malty - A***
Fruity/Estery - F***
Alcoholic/solvent - F***
Fruity/citrus - F*
Malty - F***
Other Sulfur - F**
Sweet***
Sour***
Body***
Linger*
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0% A
0% B
0% C
5% A
5% B
5% C

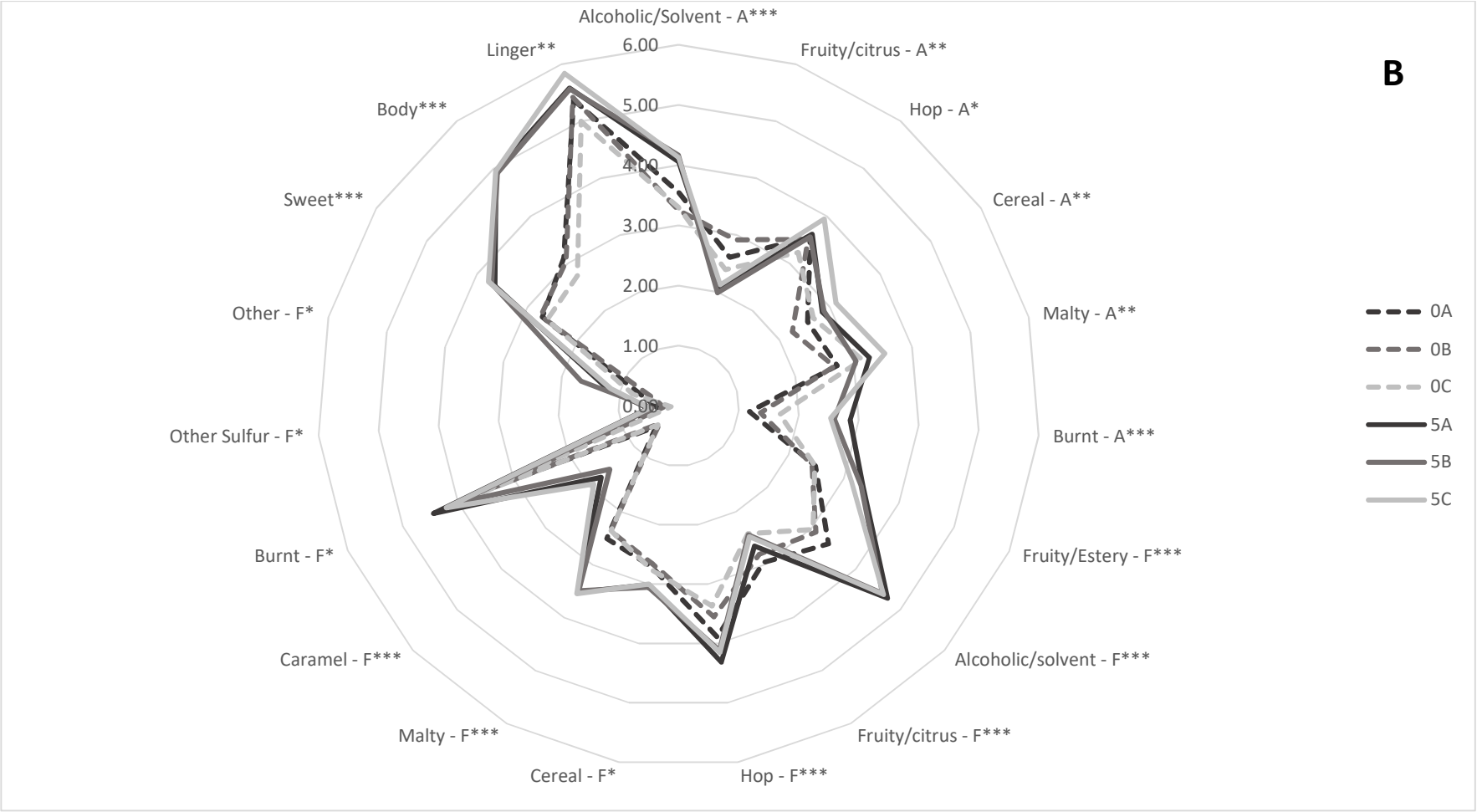


Figure 5.4: *Spider plot of mean significant sensory attribute intensities from QDA trained panel data for (A) Lager (B) Stout. Terms with '– A' after are aroma, and terms as '– F' are flavour attributes. Terms with *** are significantly different between products at p<0.0001; **p<0.01, *p<0.05*

5.4.4 Correlation between Physicochemical and Sensory Results

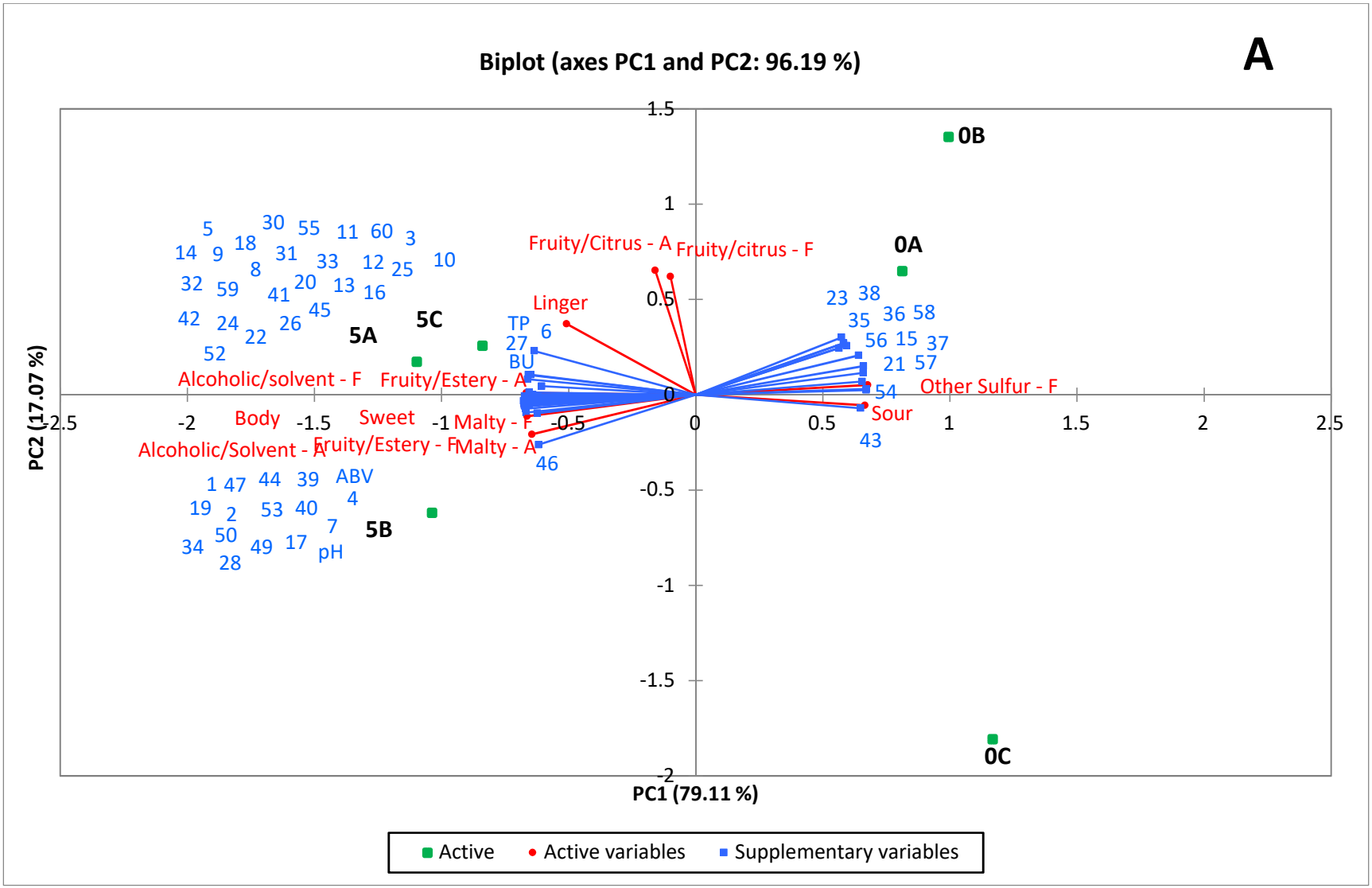
5.4.4.1 Lager

All significant physicochemical and sensory results were used to create a PCA plot (figure 5.5a). The first two principal components (PCs) of the model accounted for 96.19% of variation in the data. Most of the variance (79.11%) was explained by the first principal component (PC1) which was positively correlated with the sensory attributes 'other sulfur flavour' (0.975) and 'sour' (0.980), and compounds 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol (0.884), methyl decanoate (0.963), 3,7-dimethyl-1,6-octadien-3-ol (0.855), methyl octanoate (0.971), 2-methylbutanoic acid (0.955), benzene (0.989), 1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (0.871), terpin-4-ol (0.855), 1-methyl-4-(propan-2-yl)benzene (0.943) and (1R,4S,6S)-4,7,7-trimethylbicyclo[4.1.0]hept-2-ene (0.986). PC1 was negatively correlated with all other sensory attributes and physicochemical properties (all attributes and properties <-0.749). PC2 (showing 17.07% variation in data) was strongly positively correlated with 'fruity/citrus aroma' (0.961) and 'fruity/citrus flavour' (0.915). A significant difference between the first two trials (A and B) compared to the third trial (C) was clearly shown, with sample C positioned in the lower quadrant.

5.4.4.2 Stout

The PCA for stout samples (figure 5.5b) showed again most of the variation in the first two PCs (92.16%). PC1 (80.65%) was strongly positively correlated with nearly all sensory attributes and physicochemical properties (>0.900), apart from 'fruity/citrus aroma' (-0.895) and pH (-0.949), 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol (-0.881), methyl decanoate (-0.951),

3,7-dimethyl-1,6-octadien-3-ol (-0.892), methyl octanoate (-0.987), 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane (-0.861), 2-methylbutanoic acid (-0.935), benzene (-0.989), 1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (-0.907), terpin-4-ol (-0.893), 1-methyl-4-(propan-2-yl)benzene (-0.974) and (1R,4S,6S)-4,7,7-trimethylbicyclo[4.1.0]hept-2-ene (-0.984) which were negatively correlated. PC2 (11.51%) was strongly correlated with 'fruity/citrus flavour' (0.804) and furan-2-carbaldehyde (0.612) and negatively correlated with acetaldehyde (-0.775). As with the lager, a significant difference between the first two trials (A and B) compared to the third trial (C) was shown.


Biplot (axes PC1 and PC2: 96.19 %)
A
0B
0A
0C
5A
5B
5C
Fruity/Citrus - A
Fruity/citrus - F
Linger
TP
BU
Alcoholic/solvent - F
Fruity/Estery - A
Other Sulfur - F
Body
Sweet
Malty - F
Sour
Alcoholic/Solvent - A
Fruity/Estery - F
Malty - A
ABV
pH
PC2 (17.07 %)
PC1 (79.11 %)
Active
Active variables
Supplementary variables

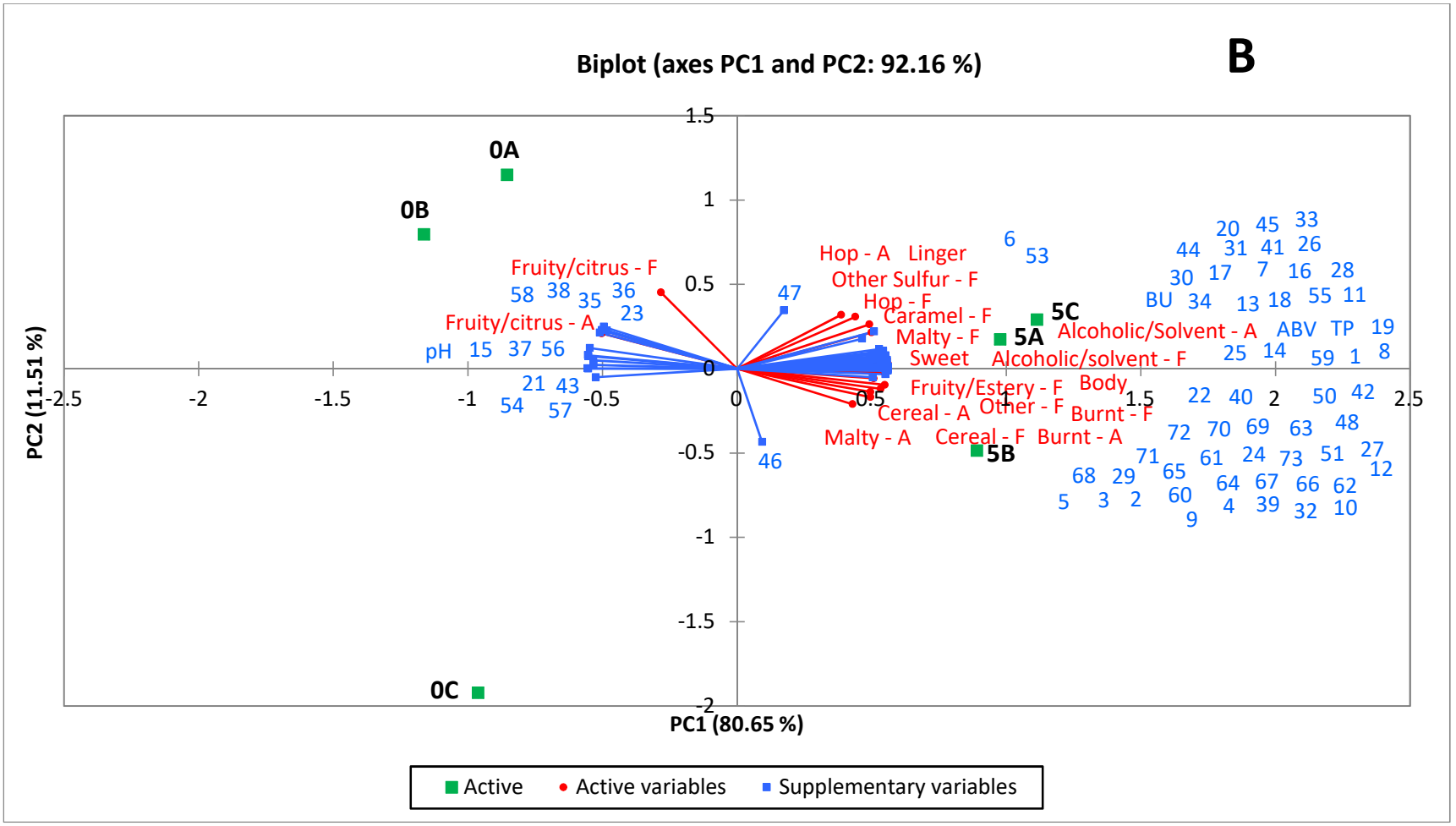


Figure 5.5: *Principal component analysis (PCA) biplot of significant physicochemical properties and sensory attributes present on principle component 1 and 2 by the covariance of means across A) Lager and B) Stout samples. Green shows the 6 samples analysed, with sensory attributes shown in red and physicochemical properties in blue. The numbers in blue correspond with the volatile compound numbers shown in brackets in Table 5.5.*

5.5 Discussion

5.5.1 Key Physicochemical and Sensorial Changes of Dealcoholised Beers as a Result of Reverse Osmosis

For the first time an understanding of the impact of reverse osmosis on the physicochemical and sensorial properties of NAB is discussed in detail, by comparing the original 5% ABV beers and their dealcoholized counterparts. Overall, data clearly showed that there were key volatile losses for both lager and stout trials resulting in changes to the sensory profile.

Although ethanol was removed by a minimum of 91% in the present study, there was also a significant reduction in many other important beer properties, including bitterness units, total polyphenols and key esters and higher alcohols. Other compounds such as carboxylic acids, aldehydes, ketones, lactones, hydrocarbons, diols and alkyl sulphides were also significantly reduced. This agreed with previous research conducted by Kavanagh et al. (1991) who used very similar techniques to dealcoholize a starting beer of 4.9% v/v to 1.0% v/v, which resulted in large losses of volatiles (77% total esters, 68% total higher alcohols). These results however, seemed to be lower than the present study, but this could have been attributed to a lower beer feed temperature (5°C), which may have reduced volatile losses (Alcantara et al., 2016).

It has previously been hypothesised that compounds with a similar structure and molecular weight to ethanol would be removed during membrane dealcoholisation, whilst anything more complex would be retained (Ben-David et al., 2006, Catarino et al., 2006, Falkenberg, 2014, Schutte, 2003). All

compounds detected in this study had a higher molecular weight than ethanol and therefore theoretically should have been rejected by the membrane, yet some were removed by up to 100%. When considering esters and higher alcohols, removal appeared to increase with increasing size (e.g ethyl acetate up to ethyl decanoate), contradicting this hypothesis and results from previous reported studies. No trend in terms of Log*P* values, a measure of polarity of the compound, were found to explain this. Key smaller esters and higher alcohols present in beer (3-methylbutyl acetate, 2-methylpropyl acetate, 3-methyl-1-butanol and 2-methylpropan-1-ol) were found to be compounds with the highest retention, and this was believed to be due to the additional methyl group within these molecules increasing branching, as well as decreased solubility in water (Falkenberg, 2014, Schutte, 2003). Overall, it appeared that compounds removed at a high level were relatively linear molecules, with low levels of branching. Other compounds which had increased branching or the presence of a benzene ring were retained, suggesting that chemical structure was important. This was confirmed using PLS-R analysis with MOE, showing that surface area + volume + shape are the key drivers of the effect, a factor which has not been used to explain this phenomenon before. Suggestions for further work are to understand this concept in more detail, by selecting key marker compounds with different structural properties and spiking them into the beer before RO dealcoholisation. This work could further understanding for brewers on which compounds are removed to a higher degree during RO and thus which ones should be focused on when producing the standard strength beer to dealcoholize.

In addition, for the first time the effect of RO on sensorial properties of beer is reported. Ethanol has been found in previous research to enhance the perception of fruity flavour, alcoholic/solvent, sweetness and fullness/body (Clark et al., 2011a, Langstaff et al., 1991, Martin and Pangborn, 1970, Ramsey et al., 2018, Williams and Rosser, 1981), with previous research also showing that RO removes volatiles that contribute to these attributes (e.g esters contributing to fruity flavour) (Alcantara et al., 2016, Catarino et al., 2007, Kavanagh et al., 1991), thus the significant attributes found in the present study confirm these findings. 'Malty aroma' and 'malty flavour' was also found to be significantly higher in the 5% beers here, which has previously been found to be the dominant attribute in regular beers before swallowing (Missbach et al., 2017), as well as a driver of consumer liking in combination with the attribute 'sweet' (Porretta and Donadini, 2008, Ramsey et al., 2018). Sensory perceptions of 'body' were also found to be significantly lower in the dealcoholised samples (both lager and stout) suggesting that mouthfeel enhancers, such as sugars were removed by the membrane due to their molecular size (Müller et al., 2017).

Overall, it was clear that the 5% sample had increased amounts of volatile flavour compounds and sensory attributes compared to the 0%, showing that there are extreme losses when subjecting a beer to RO dealcoholisation procedures. Suggestions to improve the final product and increase its comparability with the 5% beer therefore include; altering the brewing process to account for volatile aroma losses later on down the line, using special yeasts which can produce higher levels of higher alcohols and

esters during fermentation, changing the composition of brewing raw materials or selecting an RO membrane with a different composition.

5.5.2 Matrix-Membrane Interactions

Overall it was shown that RO removed key components of both beer styles, but some differences were found between the lager and stout in terms of product matrix interactions and the RO membranes. The sensory data showed the 0% lagers were perceived to be significantly more ‘sour’ and have increased ‘sulfur flavour’ compared to the 5% lager, yet these attributes were not found to be significantly different for the stouts. Previous research suggested that physical dealcoholisation techniques can produce a beer that is unbalanced in flavour, with significant increased perceived acidity due to removal of key esters and higher alcohols (Müller et al., 2017), denoting why the 0% lager may have been perceived as more sour here. The perceived increase of sulfur flavours within the 0% lagers could also simply be due to the lack of other flavours which normally work synergistically to cover up such ‘off-flavours’ (Kaipainen, 1992), yet this may not have been shown in the stout due to increased amounts of other flavour compounds (such as pyrazines and furans). No volatile compounds were identified to correlate to the attribute of ‘sulfur flavour’, but it is believed this may have been due to the increased presence of highly odour active compounds at very low concentrations within the 0% beers, which were not discovered in GC-MS analysis. This could include sulfur compounds relevant in beer including: dimethyl sulfide (DMS), dimethyl disulfide, dimethyl trisulfide and sulfur dioxide.

In addition, stout trials took significantly longer to dealcoholize due to a slower flow rate through the membrane. It is considered that this could have

been due to the starting raw materials of the stout, which contained five different malts, as well as flaked oats and roasted barley adjuncts. These previously have been found to clog membranes due to residual high molecular weight β-glucans (Briggs et al., 2004). Therefore suggestions could be made to select beers for membrane filtration made without adjuncts, to ensure less membrane clogging, quicker processing times, as well as lower production costs (Falkenberg, 2014). On the other hand, it is important to note that adjuncts such as oats can be used to improve mouthfeel (Lyly et al., 2003), which is often found to be lacking in dealcoholised beers, and hence this needs to be factored in when formulating a new NAB. However, here perceptions of 'body' were significantly reduced in the dealcoholised stout, suggesting that these were removed by the membrane. Consequently, suggestions for further work are to explore the use of adjuncts to produce more acceptable NABs, in comparison to the addition of mouthfeel enhancers at the end of membrane dealcoholisation procedures, to avoid membrane clogging whilst maximising body perception.

5.5.3 Membrane Efficiency

RO membranes can be expensive to purchase and therefore understanding their capabilities and efficiency is important for breweries. This is assessed by the quality and consistency of the finished product through replicate trials, as well as understanding indicators showing that the membrane may need to be replaced. Here three replicate trials were conducted for each beer style to further understand this.

Trial 0C for both beer styles showed differing physicochemical and sensory results compared to the two previous trials (0A and 0B) and was

positioned separately on the PCAs, showing that subsequent trials produced different results to the first. It appeared that more volatile losses occurred for trial 0C in both beer styles, with changes in sensory properties including decreased levels of ‘cereal’, ‘malty’ and ‘burnt’ aromas in 0C for the stout. This could however, have been due to the different starting volume of the stout (18L for 0C, 50L for 0A and 0B) influencing the differences in sensory attributes. Previous RO research discussed changes in subsequent trials to be due to a loss of selectivity within the membrane, indicating clogging of membrane pores, fouling or membrane cake build up (Falkenberg, 2014). Here it is believed that there was severe fouling of the membrane, meaning that certain compounds caused a blockage of the membrane pores making it difficult for ethanol to pass through into the permeate during trials, thus slowing down flow rates. Previous research has also assessed fouling coefficients of an RO membrane in a stout style beer using different diafiltration procedures (continuous and discontinuous), and found that diluting beer before dealcoholisation, rather than after, could reduce fouling by almost half (Alcantara et al., 2016). It should be highlighted however, that this previous study was only assessed in lab-scale settings with smaller starting quantities of beer (500 mL) and therefore one suggestion for further work is to understand whether the same effect is shown with larger volumes of beer using a pilot-scale dealcoholisation unit, similar to that used in the current study.

In addition, during physicochemical analysis, an increased amount of some volatile compounds were discovered in all 0% samples compared to the original 5% beers. The discovery of this taint was unusual, as the starting beers either contained a very low level of these compounds or none at all. These

compounds included certain terpenes and higher alcohols (including 4,7,7-trimethylbicyclo[4.1.0]hept-2-ene, 1,7,7-trimethylbicyclo[2.2.1]heptan-2-one, 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol, 3,7-dimethyl-1,6-octadien-3-ol and 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane), with similar molecular weights (154.25g/mol), as well as 2-methylpropyl ethanoate. The presence of these compounds also became apparent in the sensory results, with higher ratings for 'fruity/citrus aroma' and 'fruity/citrus flavour' in the 0% samples for both beer styles. Panellists described this in additional comments as 'ginger/orange/herbal/citrus' aroma and flavour. It should be noted here, that the presence of this taint did not seem to effect other sensory results, with small differences still discovered between the 0 and 5% beers. Delving deeper into the results, it was clear to see that this phenomenon was limited to a small group of volatile compounds, which all had a similar cyclic structure. It is therefore believed that these could have been adsorbed within the membrane during preceding projects, where products known to contain some of these compounds were dealcoholised using the same membrane and system. Previous research also found similar results, with linear compound structures more permeable to the membrane with an easier passage, whereas cyclic structures entered the membrane during cross filtration and then became stuck (Falkenberg, 2014). It is believed that this taint was therefore part of a contamination residue on or within the membrane, with these compounds being pulled through the membrane into the 0% beer when dealcoholisation took place. Consequently, the third trial (C) was a 'cleaner' replicate, as most of the contamination residue from the taint had been removed during the first two trials (A and B). This was revealed in the sensory data for the lager, as trial 0C

had significantly lower amounts of ‘fruity/citrus aroma’ and ‘fruity/citrus flavour’ compared to trials 0A and 0B. With this residue being removed in the third trial (0C) however, it also meant that more of the key volatiles could be removed from the beer making their way through to the permeate as ‘waste’, which was shown by increased losses of volatiles in this replicate. Again this could however, have also been down to the different starting volumes for 0C lager and stout (44.2L for lager and only 18L for stout), meaning that less processing time was needed. Many of these compounds were insoluble in water and therefore cleaning with water and NaOH (as suggested by the membrane supplier) may not have removed all traces. Therefore the importance of thorough cleaning of all kit is highlighted here. In addition, it is recommended that a separate membrane be used for different starting product matrixes.

Overall this research showed that using RO as a membrane filtration technique can produce a NAB with reduced physicochemical and sensory attributes compared to its standard alcohol counterpart. Contrary to previous findings, compound structure appeared to be more important than size when suggesting the mechanism for compound removal by RO membranes. Further improvements to the process, as well as increased understanding of product matrix interactions, are needed to produce a more acceptable NAB for consumers.

5.6 Conclusion

This study evaluated the impact of reverse osmosis on the physicochemical and sensory properties of two different beer styles (lager and stout). Results showed that there was clear differentiation between a standard alcohol beer and its lower alcohol counterpart, with severe removal of

numerous volatile compounds, including a 70% reduction in 3-methyl-1-butanol and 92% reduction in ethyl hexanoate resulting in a change in sensory properties. Dealcoholized beers had a decreased presence of the sensory attributes ‘fruity/estery aroma’, ‘alcoholic/solvent aroma’, ‘malty aroma’, ‘fruity/estery flavour’, ‘alcoholic/solvent flavour’, ‘malty flavour’, ‘sweetness’ and ‘body’. Removal of volatile compounds by the RO membrane was found to not be due to molecular size, but instead due to molecular structure with compounds with increased levels of branching (including 3-methylbutyl acetate, 2-methylpropyl acetate, 3-methyl-1-butanol and 2-methylpropan-1-ol) retained to a higher degree in comparison with more linear structured compounds. This was confirmed using molecular operation environment descriptors, which showed that surface area + volume + shape were the key drivers of the effect. The interactions between RO membranes and different product matrixes were also reported, with more sensorial differences discovered between the 0% and 5% lagers compared to the stout. This showed that dealcoholizing a lager may face increased challenges as the removal of volatiles leads to a lack of other flavours, which normally work synergistically to cover up ‘off-notes’ such as ‘sour’ taste and ‘sulfur flavour’. However, stouts present more of a challenge in terms of membrane clogging as they contain greater higher molecular weight compounds which have increased branching or ring structures. It was also noted that deep cleaning of the membrane between trials is required, as well as the use of separate membranes for different product matrixes to avoid product contamination associated with membrane fouling resulting in taints.

This research is important for the international brewing industry as the global demand for NAB is increasing rapidly. This research helps further knowledge of RO as a technique to produce NABs by reporting results from replicate trials, as well as results using different product starting matrixes, which can help breweries understand if this is a good investment for their company.

6 Conclusions and Further Work

The main objectives of this research were to develop an understanding and improved quality of NAB, using sensorial and analytical techniques. A thorough literature review was undertaken in Chapter 1, which included an overview of standard beer, its position in the market and the brewing process. NAB and the rise in market value as well as different production methods were also discussed in detail. The physicochemical and sensorial effects of ethanol were reviewed to highlight the gaps in research.

The lack of robust sensory data regarding the impact of ethanol on beer flavour perception, the sensory quality of commercial NABs and the use of membrane technologies to dealcoholise different beer styles was apparent from the literature, and therefore addressed in this thesis alongside physicochemical data.

Research in Chapter 2 found that ethanol concentration (0, 0.5, 2.8 and 5% ABV) had an effect on not only the temporal perception of attributes, but also on consumer liking. The 0 and 5% beers were found to be perceptually different by consumers, with the 5% beer perceived to be sweeter, with increased fullness/body and alcohol warming sensation. Temporality of sensory attributes was also shown, with tingly sensation one of the first attributes to appear, whilst delayed onset attributes included alcohol warming sensation, bitterness and malty and hoppy flavours. Three clusters of consumers were found, with different patterns of liking. One cluster liked the high ethanol beer (5%) the most, whereas another preferred the low/no ethanol beer samples (0%). Interestingly the largest cluster consisted of consumers who

did not show any preference for any of the samples, and this cluster was found to like malty flavour, sweet taste and alcohol warming sensation and dislike astringent and tingly sensation. These findings show that ethanol is a complex stimulus which can effect numerous sensory properties within a beer matrix. It also indicated that simply altering the ethanol concentration of a beer can have a significant impact on consumer overall liking, however this impact can be positive or negative dependent on the individual consumer. This information is useful for brewers as it highlights certain groups of consumers that can be targeted, with brewers developing the ideal product for a cluster of consumers, avoiding attributes found to drive consumer disliking and focusing on attributes which drive liking. The mechanisms for the results found here could be due to changes in the physicochemical matrix with the addition of ethanol, in-mouth interactions or multimodal flavour perception.

Therefore, research conducted in Chapter 3 aimed to explore the effect of ethanol concentration on volatile aroma release and how this influenced sensory changes. Research conducted confirmed that ethanol had an effect on volatile aroma release, which in turn influenced sensory perception. Although consumers could not discriminate between ethanol concentrations (0 and 5%) orthonasally, in-mouth flavour assessments showed significant differences. The 0% beer was perceived to be maltier with reduced fruitiness, sweetness, fullness/body and alcohol warming sensation. This was proposed to be due to the presence of saliva during ingestion, as well as ethanol interacting multimodally with gustatory, olfactory and trigeminal modalities. In vitro assessments using GC-MS showed the headspace intensity of aroma compounds was lower in the 5% beer compared to the 0%, whilst discovering

an effect of product matrix, with aroma release in a stout lower in comparison to a lager due to a higher macromolecular content. The presence of α-amylase (salivary protein) was found to have an effect in vitro, dependent on aroma compound hydrophobicity. A shift was discovered in the presence of higher ethanol concentrations to more hydrophobic compounds such as ethyl hexanoate and linalool. This was proposed to be due to hydrophobic interactions, resulting in aroma compound and salivary protein binding. Molecular hydrodynamics was also applied to discover that the salivary protein was denatured in the presence of higher concentrations of ethanol (5-20% ABV), changing from globular to elongated structures. These changes were suggested to be strongly correlated with the changes found in flavour and mouthfeel perception. This finding is key as it highlights the importance of linking sensory and analytical techniques to understand the effect of changes on a product matrix. The importance of understanding the interactions between the product matrix and consumption dynamics is clearly a noteworthy factor. It also provides evidence to brewers to tackle the lost functionality of ethanol in a NAB matrix.

Research in Chapter 2 and 3 clearly showed that if simple removal of ethanol were possible, there are still significant sensory and physicochemical changes that need to be addressed. However, ethanol removal is never simple, especially from a complex product matrix such as beer. Therefore, chapter 4 explored the sensory and physicochemical properties of commercial NABs, alongside consumer liking, with exploration into the importance of production method. Results identified a range of commercial NABs with identifiable characteristics, which could not be explained by production methods, showing

that this is not the main factor affecting the overall sensory quality of the beers but instead could be due to different starting raw materials or post processing methods. Overall, consumers liked samples with what could be described as a 'bland' flavour profile, as none of the sensory attributes were rated highly for these beers. This could also show that these samples were well balanced. Interestingly the most liked samples by consumers were those that were produced using mixed methods (biological and physical production methods). Beers with flavour profiles of initial and lingering bitterness and astringency were found to be the least liked. Cluster analysis showed that there were five different clusters of consumers found with different likes/dislikes. As with the study performed in Chapter 2, it was clear that there were individual differences within a population. Beers with strong flavour profiles such as 'hoppy' were either enthusiastically liked or disliked by certain consumers showing the need for a diverse range of NAB within the brewing sector.

Finally, the effect of one selected production method, reverse osmosis, was explored in further detail in Chapter 5. This research confirmed that RO had a significant impact on the overall quality of both lager and stout style beers, assessed through sensorial and analytical techniques. Both standard lager and stout style beers were dealcoholized in replicate trials using a pilot-scale dealcoholisation unit. A deeper understanding into the sensorial and physicochemical losses attributed to RO dealcoholisation technology were described, which have not previously been reported in scientific literature. Unsurprisingly, results showed that the standard beers and their dealcoholized counterparts were significantly different from each other for both beer styles, due to extreme losses of volatile flavour compounds effecting sensorial

characteristics. When comparing the two different beer styles, it was found that the NA lager was perceived to be significantly more sour and had increased sulphur flavour when compared to the higher alcohol counterpart, and it was concluded that compounds which normally work synergistically to cover up 'off-flavours' in the lager were removed. The stout did not show these changes and it was believed this was due to increased amounts of other flavour compounds such as pyrazines and furans hiding these 'off-flavours'. In addition, the stout was found to take a significantly longer duration to dealcoholize, with increased membrane fouling due to higher molecular weight compounds being present. Finally, interactions with certain volatile compounds and the RO membrane occurred and it was believed this was due to their cyclic structure adsorbing to the membrane in preceding trials and being released in further trials. The results of this can be used to help breweries tackle the challenges of the removal of ethanol through RO and could help guide breweries in the development of a beer with higher levels of compounds that were discovered to be removed at higher levels.

Overall, it is hoped that findings from this thesis can be applied in real world situations in the brewing industry, to help improve the overall acceptability of NABs within the market. This thesis has offered results to unanswered questions in the development of NAB, and further work should look to explore the following:

- Following on from findings that the beer matrix (lager vs stout) impacts the quality of NAB produced by membrane filtration techniques, further work should explore changes in sensory properties with other product matrixes (e.g ales, sours, wheat

beers) made by different production techniques (e.g biological and physical techniques).

- Clustering techniques employed in Chapter 1 showed that there were different liking patterns amongst consumers. Therefore recruiting these different types of consumers in sufficient numbers would allow understanding of consumer liking of beer at different ethanol concentrations to be gathered.
- Different methods of measuring volatile aroma release can give insightful results on the dynamics of consumption, shown in Chapter 2. Other in-vivo techniques such as the use of APCI-MS, as well as the capturing of volatile release in mouth after consumption using either SPME fibres or BioVOC breath samplers (Markes International, UK) can show a deeper insight into the changes of ethanol concentration within a beer matrix. Previous studies have conducted similar techniques using different wines (red, white and a model wine) (Muñoz-González et al., 2014a) yet no research to date has looked at these differences amongst beer styles.
- A further understanding on NAB production methods could be gained by future research using the same starting beer for a number of production methods, so that the fundamental properties of these methods can be better understood and built upon in future years.
- Chapter 4 discovered the sensory attributes of cooked vegetable, burnt, sulphur, rubbery aroma, cardboard flavour and

metallic, however these were not correlated to any flavour compounds reported. It was proposed that this could be due to the presence of highly odour active compounds, such as sulphurs, at very low concentrations, which could not be identified through the GC-MS analysis. Further work utilising a flame photometric detector or sulphur chemiluminescence detection is therefore suggested to understand the presence of these sulfur compounds in NABs and their contribution to these negative sensory attributes.

- Predictive modelling is a beneficial technique to predict the behaviour of flavour molecules and their interaction with RO membranes. PLS-R modelling was used in Chapter 5 to explain the trapping of particular molecules on the membrane using molecular descriptors. Further insights using this technique would be beneficial for the brewing sector, as it could guide brewers on starting recipes for beers before dealcoholisation using RO.
- Exploration of novel ways to counteract the lost functionality of ethanol sensorially and analytically (found in Chapters 2 and 3) would be beneficial for brewers embarking on improving their NAB profile. Chapter 2 found that the reduction of ethanol decreased the citation of sensory attributes such as alcohol warming sensation and mouthfeel/body, yet these attributes were key drivers of liking for different clusters of consumers.

With the optimisation of these attributes, consumer liking could be increased in this sector.

- Psychological aspects of NAB such as emotional response and consumer perception are interesting areas to explore in this sector, as previous research has shown consumer expectations of NAB are not fulfilled (Chaya et al., 2015, Silva et al., 2016, Silva et al., 2017). To ensure commercial success of NAB, it would be interesting to observe differences in consumer responses to different marketing efforts (e.g NAB or functional drink).
- In addition to this, intrinsic (e.g sensory and physicochemical properties presented in this thesis) and extrinsic (e.g health claims, price, design, packaging) (Blackmore et al., 2020, Silva et al., 2017) product cues have been researched separately, yet few studies have looked at these together. In the study by Blackmore et al. (2020) it was found that labelled alcohol content altered expectations of bitterness, body and beer colour, yet this had no effect on consumer liking, a surprising finding. From the results found in Chapter 2, it was clear that alcohol content had an effect on consumer liking, yet these samples were served blind to consumers. Therefore it would be highly useful to combine both techniques to give an overall view on consumer perception of products before consumption using extrinsic cues, and then understand whether this changes after consumption with intrinsic cues.

Appendices

Appendix Table 1: *Effect of ethanol and α-amylase on a) lager style beer; b) stout style beer. Values in bold are significant at p<0.05.* [abcd]*Different letters within a column represent significant differences among samples.*

A - Lager	*Furfural*	*Ethyl Acetate*	*3-Methylbutanal*	*Isoamyl Alcohol*	*Phenylethyl Alcohol*	*Hexanal*	*Isoamyl Acetate*	*Ethyl Hexanoate*	*Linalool*
0% Ethanol	6.74E+07[a]	2.79E+09[a]	3.98E+08[a]	2.24E+09[a]	2.69E+08[a]	1.55E+08[a]	2.79E+09[a]	5.46E+08[a]	6.36E+07[a]
0% Ethanol + α-amylase	5.86E+07[a]	2.97E+09[a]	3.16E+08[b]	2.10E+09[a]	2.32E+08[ab]	1.05E+08[b]	1.97E+09[c]	2.17E+08[c]	2.47E+07[b]
5% Ethanol	4.25E+07[a]	1.33E+09[b]	1.92E+08[c]	1.44E+09[b]	2.18E+08[ab]	9.79E+07[bc]	2.30E+09[b]	4.39E+08[b]	4.54E+07[ab]
5% Ethanol + α-amylase	3.91E+07[a]	1.32E+09[b]	1.42E+08[d]	1.36E+09[b]	1.69E+08[b]	7.34E+07[c]	1.72E+09d	1.97E+08[c]	2.31E+07[b]
p values	0.059	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.014**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.004**

B - Stout	*Furfural*	*Ethyl Acetate*	*3-Methylbutanal*	*Isoamyl Alcohol*	*Phenylethyl Alcohol*	*Hexanal*	*Isoamyl Acetate*	*Ethyl Hexanoate*	*Linalool*
0% Ethanol	5.00E+07[a]	1.02E+09[a]	5.09E+08[a]	1.65E+09[a]	1.51E+08[a]	1.79E+08[a]	8.10E+08[a]	4.39E+08[a]	7.40E+07[a]
0% Ethanol + α-amylase	5.61E+07[a]	1.10E+09[a]	4.27E+08[b]	1.61E+09[a]	1.26E+08[a]	1.05E+08[b]	5.52E+08[b]	1.82E+08[c]	3.17E+07[c]
5% Ethanol	3.20E+07[b]	3.69E+08[b]	2.22E+08[c]	9.53E+08[b]	1.26E+08[a]	1.07E+08[b]	4.84E+08[bc]	2.93E+08[b]	4.80E+07[b]
5% Ethanol + α-amylase	3.26E+07[b]	3.77E+08[b]	1.74E+08[d]	8.98E+08[b]	1.12E+08[a]	6.03E+07[b]	3.55E+08[c]	1.33E+08[c]	2.56E+07[c]
p values	**0.000**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.145	**0.000**	**< 0.0001**	**< 0.0001**	**< 0.0001**

Appendix Table 2: *CATA (orthonasal aroma) and TCATA (in-mouth flavour, taste and mouthfeel) attributes and definitions provided to consumers during familiarisation session.*

	Attributes	**Definition**
Aroma	*Fruity*	Smell of fruits such as banana, green apple, pineapple, peach, lemon, lime, orange or grapefruit.
	Malty	Small of cereals or grains. Can be related to smell of Ovaltine drink.
	Hoppy	Smell of hops, which can be floral/herbal.
	Stale	Musty smell or smell of wet paper/cardboard.
	Cooked Vegetable	Smell of cooked vegetables such as cabbage or sweetcorn. Can also be related to a sulphur smell.
	Alcohol	Smell of alcohol/spirits.
Flavour and Taste	*Malty Flavour*	Flavour of malty cereals. Can be related to smell of Ovaltine drink.
	Hoppy Flavour	Flavour of hops which can be flowery and herbal.
	Fruity Flavour	Flavour of fruit characteristics – including banana, apple, pineapple, peach, lemon, orange.
	Bitter Taste	Taste stimulated by strong black coffee, beer, red wine or tonic water.
	Sweet Taste	Taste stimulated by sugar when experienced in mouth.
	Sour Taste	Taste stimulated by acids when experienced in mouth.

Mouthfeel	*Fullness/Body*	Feeling of thickness/fullness as beer is moved around in the mouth.
	Alcohol Warming Sensation	The feeling of warming which is characteristic of ethanol throughout the mouth.
	Tingly Sensation	Perception of irritation such as prickling, stinging and bubbles bursting in mouth from carbonation. The feeling of pins and needles.
	Astringent Mouthfeel	The feeling in mouth of roughing, puckering and drying.

Appendix Table 3: *Attributes, definitions and reference standards used in QDA trained sensory panel (n=10)*

	Attribute	Description	Reference
Aroma	Cooked Vegetables	Aroma associated with overcooked green vegetables such as cabbage, broccoli or Brussel sprouts or tinned sweetcorn (DMS)	20 mL water from overcooked boiled cabbage; 150 ug DMS/L beer (AROXA™)
	Rubbery	Aroma associated with rubber car tyres	N/A
	Sulphur	Aroma associated with a struck match	21 mg sulphur dioxide/L beer (AROXA™)
	Grassy/Green	Aroma associated with freshly cut grass or chopped leaves	2.9 mg cis-3-hexenol/L beer (AROXA™)
	Banana/Pear Drops	Aroma associated with ripe or artificial banana and pear drops	3.5 mg isoamyl acetate/L beer (AROXA™)
	Tropical Fruits	Overall intensity of aroma associated with tropical fruits including pineapple, mango, passionfruit and peach	20 mL tropical fruit juice
	Floral	Aroma associated with flowers, particularly roses or violets	4 mg B-iodine/L beer (AROXA™); 1.2 mg geraniol/L beer (AROXA™)
	Grainy	Aroma associated with whole raw barley grain and hay/straw	10 g raw barley grain
	Burnt	Aroma associated with burnt toast, dark roasted malt or burnt sugar (treacle)	10 g black treacle (Tate and Lyle)
Flavour	Banana/Pear Drops	Flavour associated with ripe or artificial banana and pear drops	3.5 mg isoamyl acetate/L beer (AROXA™)
	Grapefruit	Flavour associated with freshly cut white grapefruit	5 g freshly cut white grapefruit flesh and skin
	Hoppy	Flavour associated with fresh hops crushed in hand or hop pellets	1.25 mg hop oil extract/L beer (AROXA™)
	Malty	Flavour associated with malt extract and fresh wort, which may also contain caramel notes	50 g malt extract mixed with 50 mL water; 20 mL fresh lager wort

	Cardboard	Flavour associated with damp cardboard	5 g cardboard in 10 mL water
	Yeasty	Flavour associated with rehydrated yeast or bread dough	5 g bread yeast (Allinson) in 10 mL water; bread dough
Taste	Initial Bitterness	Taste stimulated by bitter substances such as caffeine or quinine	13 ul 30% iso-α-acids (TNS®) in 330 mL water
	Sweet	Taste stimulated by sucrose	8.5 mg sucralose/L beer (AROXA™)
	Sour	Taste stimulated by acids	457 mg citric acid/L beer (AROXA™)
	Lingering Bitterness	Persistence of bitterness in mouth, perceived 20 seconds after swallowing	13 uL 30% iso-α-acids (TNS®) in 330 mL water
Mouthfeel	Thick/Full	Perception of thickness/fullness and syrupy mouthcoating, as beer is moved around in mouth.	N/A
	Metallic	The taste of blood or iron, perceived 20 seconds after swallowing	8.2 mg ferrous sulphate/L beer (AROXA™)
	Peppery	The perception of heat/chilli in back of throat and tip of tongue, perceived 30 seconds after swallowing	20 mL ginger beer (Old Jamaica)
	Astringent	The feeling of drying/mouth puckering in mouth after swallow, perceived 30 seconds after swallowing	1% tannic acid solution in water

Appendix Table 4: *Mean intensity of significant aroma, flavour, taste and mouthfeel attributes as evaluated by trained QDA panel.*

Different letters within a column represent a significant difference among samples based on differences in HSD ($p<0.05$)

	Aroma									Flavour						Taste				Mouthfeel			
Sample Name	Cooked Vegetables	Rubbery	Sulphur	Grassy/Green	Banana/Pear Drops	Tropical Fruits	Floral	Grainy	Burnt	Banana/Pear Drops	Grapefruit	Hoppy	Malty	Cardboard	Yeasty	Initial Bitterness	Sweet	Sour	Lingering Bitterness	Thick/Full	Metallic	Peppery	Astringent
1	1.01^{efg}	0.19^{d}	0.08^{g}	1.37^{bcde}	0.44^{bc}	1.38^{bcd}	1.69^{b}	5.45^{abc}	1.41^{def}	0.44^{d}	0.62^{bc}	1.47^{c}	7.65^{ab}	1.94^{abcde}	2.75^{abcd}	3.15^{fg}	6.29^{ab}	1.29^{ef}	3.36^{efgh}	6.11^{ab}	2.02^{def}	1.75^{b}	3.96^{efg}
2	1.34^{defg}	0.10^{d}	1.02^{defg}	1.61^{bcde}	0.17^{bc}	1.01^{bcd}	1.48^{b}	4.57^{abcd}	0.97^{def}	0.09^{d}	2.06^{b}	2.24^{bc}	4.10^{ef}	3.24^{a}	2.15^{abcde}	5.18^{bcd}	2.51^{fgh}	3.24^{bcd}	5.18^{bc}	3.70^{efg}	3.55^{bcd}	2.37^{b}	5.96^{abc}
3	4.91^{a}	5.37^{a}	5.60^{a}	0.34^{e}	0.08^{bc}	0.10^{d}	0.09^{b}	1.81^{ef}	4.31^{ab}	0.53^{cd}	0.94^{bc}	1.10^{c}	4.47^{def}	2.64^{abc}	3.56^{a}	4.62^{bcdef}	2.65^{fgh}	2.85^{bcde}	4.70^{bcde}	4.12^{defg}	4.93^{ab}	1.19^{b}	4.97^{cdef}
4	1.83^{cdefg}	1.06^{cd}	1.55^{defg}	1.67^{bcde}	1.01^{bc}	0.93^{bcd}	1.27^{b}	3.42^{cde}	2.49^{bcd}	0.38^{d}	0.79^{bc}	1.85^{bc}	5.98^{bcd}	2.09^{abcde}	3.10^{abc}	4.96^{bcde}	5.07^{bcd}	2.10^{def}	4.77^{bcde}	5.37^{abcd}	4.63^{abc}	2.21^{b}	5.03^{cdef}
5	1.84^{cdefg}	0.37^{d}	0.60^{fg}	1.04^{de}	0.01^{c}	0.80^{cd}	1.20^{b}	5.97^{ab}	1.52^{def}	0.10^{d}	0.57^{c}	1.95^{bc}	8.38^{a}	2.00^{abcde}	3.18^{abc}	2.95^{g}	7.08^{a}	1.20^{ef}	2.89^{gh}	6.73^{a}	2.60^{def}	1.37^{b}	3.45^{g}
6	0.70^{fg}	0.36^{d}	0.26^{fg}	2.96^{ab}	0.20^{bc}	5.39^{a}	4.25^{a}	1.76^{ef}	0.38^{ef}	0.32^{d}	6.00^{a}	7.20^{a}	2.07^{gh}	1.01^{cde}	1.09^{de}	4.15^{cdefg}	3.22^{efg}	3.76^{abcd}	3.93^{cdefg}	4.03^{defg}	1.11^{f}	1.19^{b}	4.29^{defg}
7	4.51^{ab}	4.39^{ab}	3.90^{abc}	0.56^{e}	0.01^{c}	0.01^{d}	0.01^{b}	1.21^{f}	4.53^{a}	0.14^{d}	1.64^{bc}	1.25^{c}	2.80^{fgh}	2.86^{ab}	1.89^{abcde}	7.90^{a}	0.86^{i}	4.42^{ab}	7.65^{a}	3.17^{fg}	5.77^{a}	0.91^{b}	6.94^{a}
8	4.03^{abc}	4.03^{ab}	4.78^{ab}	1.02^{de}	0.29^{bc}	0.02^{d}	0.16^{b}	1.74^{ef}	4.02^{abc}	0.22^{d}	1.10^{bc}	1.68^{bc}	3.49^{fg}	3.53^{a}	1.45^{cde}	8.07^{a}	0.86^{i}	3.77^{abc}	7.42^{a}	2.69^{g}	5.75^{a}	1.49^{b}	6.71^{ab}
9	1.32^{defg}	0.49^{d}	0.22^{fg}	1.19^{bcde}	7.11^{a}	2.21^{bc}	1.66^{b}	1.96^{ef}	0.44^{ef}	7.28^{a}	1.02^{bc}	1.75^{bc}	2.63^{fgh}	0.66^{e}	0.60^{e}	2.94^{g}	3.99^{def}	2.55^{cdef}	2.09^{h}	3.53^{efg}	1.34^{ef}	4.89^{a}	3.43^{g}
10	1.34^{defg}	1.54^{cd}	0.88^{efg}	1.21^{bcde}	1.40^{b}	2.49^{b}	1.71^{b}	1.57^{ef}	0.53^{ef}	1.68^{c}	1.10^{bc}	2.00^{bc}	2.79^{fgh}	1.27^{bcde}	2.04^{abcde}	3.53^{efg}	3.98^{def}	2.20^{cdef}	3.15^{fgh}	3.70^{efg}	2.38^{def}	1.65^{b}	4.41^{defg}
11	2.27^{bcdefg}	1.32^{cd}	1.20^{defg}	1.57^{bcde}	0.88^{bc}	0.51^{d}	1.77^{b}	2.69^{def}	0.99^{def}	1.71^{c}	0.76^{bc}	1.43^{c}	3.69^{efg}	1.89^{abcde}	1.52^{cde}	4.55^{bcdef}	2.88^{fgh}	2.53^{cdef}	3.70^{defg}	3.09^{fg}	3.30^{bcde}	1.13^{b}	4.70^{cdef}
12	2.36^{bcdefg}	2.55^{bc}	2.06^{cdef}	2.60^{abcd}	0.59^{bc}	0.53^{d}	0.34^{b}	2.60^{def}	1.32^{def}	0.59^{cd}	1.75^{bc}	1.98^{bc}	3.56^{fg}	2.49^{abcd}	1.14^{de}	5.44^{bc}	1.46^{hi}	4.44^{ab}	5.14^{bcd}	3.26^{fg}	3.57^{bcd}	1.28^{b}	5.03^{cdef}
13	0.14^{g}	0.34^{d}	0.18^{fg}	2.87^{abc}	0.65^{bc}	6.84^{a}	4.17^{a}	1.06^{f}	0.07^{f}	0.68^{cd}	7.16^{a}	7.61^{a}	1.50^{h}	0.90^{de}	0.46^{e}	5.94^{b}	1.50^{hi}	5.14^{a}	5.81^{b}	2.57^{g}	3.16^{bcde}	1.39^{b}	5.76^{abcd}
14	2.47^{bcdef}	1.98^{cd}	2.87^{bcd}	1.44^{bcde}	0.54^{bc}	0.71^{cd}	0.43^{b}	2.19^{ef}	2.24^{cde}	0.92^{cd}	1.33^{bc}	2.12^{bc}	3.39^{fg}	2.55^{abc}	1.70^{bcde}	4.55^{bcdef}	1.76^{ghi}	4.51^{ab}	4.08^{cdefg}	3.28^{fg}	3.58^{bcd}	1.38^{b}	5.38^{bcde}
15	2.34^{bcdefg}	0.87^{cd}	0.70^{efg}	1.32^{bcde}	0.40^{bc}	1.03^{bcd}	1.00^{b}	4.29^{bcd}	1.46^{def}	0.50^{cd}	0.72^{bc}	1.52^{c}	5.56^{cde}	2.47^{abcd}	1.66^{bcde}	3.87^{defg}	4.05^{def}	2.20^{cdef}	4.09^{cdefg}	4.35^{cdef}	2.76^{cdef}	1.21^{b}	4.40^{defg}
16	1.11^{defg}	0.17^{d}	0.34^{fg}	1.59^{bcde}	6.12^{a}	1.49^{bcd}	0.96^{b}	1.73^{ef}	1.08^{def}	5.02^{b}	1.05^{bc}	1.47^{c}	3.52^{fg}	1.36^{bcde}	0.99^{de}	4.40^{cdefg}	2.77^{fgh}	2.52^{cdef}	4.36^{cdef}	3.54^{efg}	3.01^{bcdef}	1.96^{b}	5.03^{cdef}
17	3.17^{abcde}	1.87^{cd}	2.55^{cde}	1.10^{cde}	0.64^{bc}	0.42^{d}	0.32^{b}	2.91^{def}	1.77^{def}	1.14^{cd}	1.14^{bc}	1.82^{bc}	4.26^{def}	2.75^{ab}	3.38^{ab}	4.36^{cdefg}	4.49^{cde}	3.17^{bcd}	4.19^{cdefg}	4.87^{bcde}	3.75^{bcd}	1.19^{b}	4.48^{defg}
18	3.32^{abcd}	1.22^{cd}	0.63^{fg}	3.76^{a}	0.18^{bc}	1.40^{bcd}	1.80^{b}	6.36^{a}	1.34^{def}	0.25^{d}	1.05^{bc}	3.49^{b}	7.11^{abc}	2.64^{abc}	3.54^{a}	3.61^{efg}	5.94^{abc}	1.12^{f}	2.99^{fgh}	5.86^{abc}	1.99^{def}	1.57^{b}	3.86^{fg}

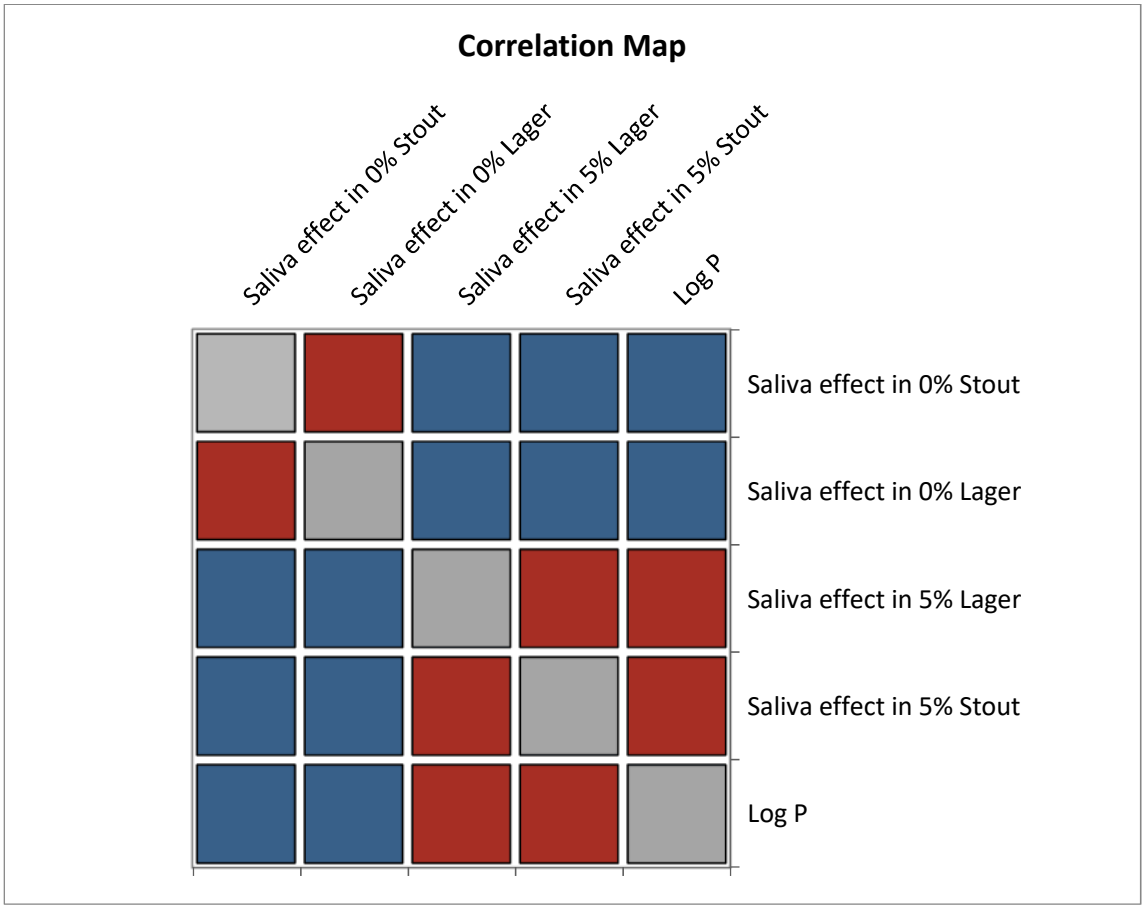
Appendix Table 5: *Peak area ratio of compounds detected by SPME-GC-MS, with corresponding retention times. Different letters within a column[abc] represent a significant difference among samples in terms of volatile concentrations (Tukey's HSD, p<0.05).*

Peak Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Name	3-Methylbutanal	Ethyl Propanoate	Methyl 2-Methylbutanoate	2-Methylpropyl 2-Methylpropanoate	2-Methylbutyl 2-Methylpropanoate	Methyl 5-Methylhexanoate	Styrene	2-Methylbutyl 2-Methylbutanoate	2-Methylbutyl 3-Methylbutanoate	cis-3-Hexenyl Acetate	Methyl Octanoate	3-Furaldehyde	Linalool	Trans-2-Nonenal	5-Methylfurfural	2-Furanmethanol	Diethyl butanedioate	Methional	2-Phenylethyl Acetate	2-Phenylethanol
Retention Time	4.66	5.35	6.37	6.52	7.80	11.25	11.84	12.01	12.30	12.83	14.22	15.93	16.98	17.24	17.90	19.07	19.30	20.11	21.80	23.20
Peak Area Ratio	0.03^{bc}	0.00^{f}	0.04^{abcd}	0.00^{ab}	0.08^{d}	0.00^{f}	0.23^{b}	0.00^{b}	0.01^{c}	0.00^{b}	0.01^{c}	0.02^{de}	0.08^{d}	0.01^{b}	0.00^{bc}	0.01^{ghij}	0.01^{b}	0.00^{c}	0.34^{c}	0.23^{ijk}
	0.01^{c}	0.00^{f}	0.04^{abcd}	0.00^{a}	0.13^{d}	0.00^{f}	0.04^{b}	0.00^{b}	0.01^{c}	0.00^{b}	0.10^{bc}	0.01^{e}	0.08^{d}	0.00^{bc}	0.00^{bc}	0.00^{k}	0.00^{b}	0.00^{c}	0.02^{f}	0.15^{jk}
	0.01^{c}	0.00^{f}	0.04^{bcd}	0.00^{ab}	0.00^{d}	0.00^{f}	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{b}	0.01^{c}	0.01^{e}	0.00^{i}	0.00^{c}	0.00^{bc}	0.01^{efghi}	0.00^{b}	0.00^{c}	0.00^{f}	0.56^{hi}
	0.02^{bc}	0.18^{a}	0.04^{abcd}	0.00^{ab}	0.04^{d}	0.00^{f}	0.01^{b}	0.00^{b}	0.01^{c}	0.00^{b}	0.00^{c}	0.05^{bc}	0.02^{fghi}	0.00^{c}	0.00^{b}	0.03^{cd}	0.00^{b}	0.00^{c}	1.04^{b}	1.30^{fg}
	0.05^{a}	0.00^{f}	0.05^{ab}	0.00^{ab}	0.17^{d}	0.00^{f}	0.01^{b}	0.00^{b}	0.01^{c}	0.00^{b}	0.00^{c}	0.07^{ab}	0.04^{e}	0.00^{c}	0.00^{bc}	0.02^{efgh}	0.00^{b}	0.00^{c}	0.00^{f}	0.02^{k}
	0.02^{bc}	0.00^{f}	0.05^{a}	0.01^{a}	10.64^{a}	0.03^{a}	0.69^{ab}	0.00^{b}	0.09^{bc}	0.00^{b}	0.01^{c}	0.01^{e}	0.17^{c}	0.01^{b}	0.00^{bc}	0.02^{efg}	0.00^{b}	0.00^{c}	0.03^{f}	0.53^{hij}
	0.01^{bc}	0.00^{f}	0.04^{bcd}	0.00^{ab}	0.00^{d}	0.00^{f}	0.05^{b}	0.00^{b}	0.00^{c}	0.00^{b}	0.01^{c}	0.01^{e}	0.00^{hi}	0.00^{c}	0.00^{c}	0.01^{ijk}	0.00^{b}	0.00^{c}	0.00^{f}	1.47^{f}
	0.02^{bc}	0.00^{f}	0.04^{abcd}	0.00^{ab}	0.00^{d}	0.00^{f}	0.07^{b}	0.00^{b}	0.00^{c}	0.00^{b}	0.32^{abc}	0.01^{e}	0.00^{i}	0.00^{c}	0.00^{bc}	0.02^{def}	0.00^{b}	0.00^{b}	0.01^{f}	2.05^{e}
	0.01^{c}	0.01^{ef}	0.04^{abcd}	0.00^{ab}	0.11^{d}	0.00^{f}	0.00^{b}	0.00^{b}	0.04^{c}	0.00^{b}	0.03^{c}	0.02^{e}	0.01^{ghi}	0.00^{c}	0.00^{bc}	0.01^{jk}	0.00^{b}	0.00^{c}	0.01^{f}	1.36^{f}
	0.02^{bc}	0.00^{f}	0.04^{bcd}	0.00^{ab}	0.09^{d}	0.01^{c}	0.39^{ab}	0.00^{b}	0.05^{c}	0.01^{b}	0.09^{c}	0.01^{e}	0.03^{ef}	0.00^{c}	0.00^{bc}	0.02^{de}	0.00^{b}	0.00^{c}	0.19^{d}	1.37^{f}
	0.01^{bc}	0.00^{ef}	0.04^{abcd}	0.00^{ab}	0.02^{d}	0.00^{de}	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{b}	0.24^{bc}	0.02^{e}	0.01^{ghi}	0.00^{c}	0.00^{bc}	0.01^{fghij}	0.00^{b}	0.00^{c}	0.29^{c}	2.26^{de}
	0.02^{bc}	0.01^{ef}	0.04^{abcd}	0.01^{a}	8.76^{b}	0.03^{b}	0.01^{b}	0.01^{a}	0.23^{b}	0.00^{b}	0.25^{bc}	0.02^{e}	0.02^{fgh}	0.00^{c}	0.00^{bc}	0.03^{bc}	0.00^{b}	0.01^{a}	0.11^{c}	2.50^{cd}
	0.03^{b}	0.01^{e}	0.05^{abc}	0.00^{ab}	3.03	0.00^{e}	1.11^{a}	0.00^{b}	0.09^{bc}	0.00^{b}	0.70^{a}	0.01^{e}	0.19^{b}	0.01^{b}	0.00^{bc}	0.02^{defg}	0.00^{b}	0.00^{bc}	0.05^{ef}	3.30^{b}
	0.01^{bc}	0.04^{d}	0.04^{cd}	0.00^{ab}	0.01^{d}	0.00^{f}	0.01^{b}	0.00^{b}	0.00^{c}	0.00^{b}	0.03^{c}	0.02^{e}	0.00^{hi}	0.00^{c}	0.00^{bc}	0.04^{a}	0.11^{a}	0.00^{bc}	0.18^{d}	5.04^{a}
	0.02^{bc}	0.00^{f}	0.04^{abcd}	0.00^{ab}	0.10^{d}	0.00^{f}	0.05^{b}	0.00^{b}	0.00^{c}	0.00^{b}	0.01^{c}	0.04^{cd}	0.02^{efg}	0.00^{c}	0.00^{bc}	0.03^{bcd}	0.00^{b}	0.00^{bc}	0.04^{f}	0.90^{gh}
	0.02^{bc}	0.10^{c}	0.04^{d}	0.00^{a}	0.03^{d}	0.00^{f}	0.03^{b}	0.00^{b}	0.00^{c}	0.00^{b}	0.21^{bc}	0.01^{e}	0.00^{i}	0.00^{c}	0.00^{bc}	0.03^{ab}	0.00^{b}	0.00^{bc}	1.43^{a}	3.39^{b}
	0.01^{c}	0.17^{b}	0.04^{abcd}	0.00^{b}	0.02^{d}	0.00^{f}	0.02^{b}	0.00^{b}	0.00^{c}	0.00^{b}	0.02^{c}	0.01^{e}	0.02^{fghi}	0.00^{c}	0.00^{bc}	0.01^{ijk}	0.00^{b}	0.00^{c}	0.02^{f}	2.82^{c}
	0.05^{a}	0.00^{f}	0.04^{abcd}	0.00^{ab}	3.10^{c}	0.00^{f}	0.01^{a}	0.01^{a}	0.53^{a}	0.47^{a}	0.45^{ab}	0.09^{a}	0.59^{a}	0.02^{a}	0.04^{a}	0.01^{hijk}	0.00^{b}	0.00^{c}	0.01^{f}	0.14^{jk}

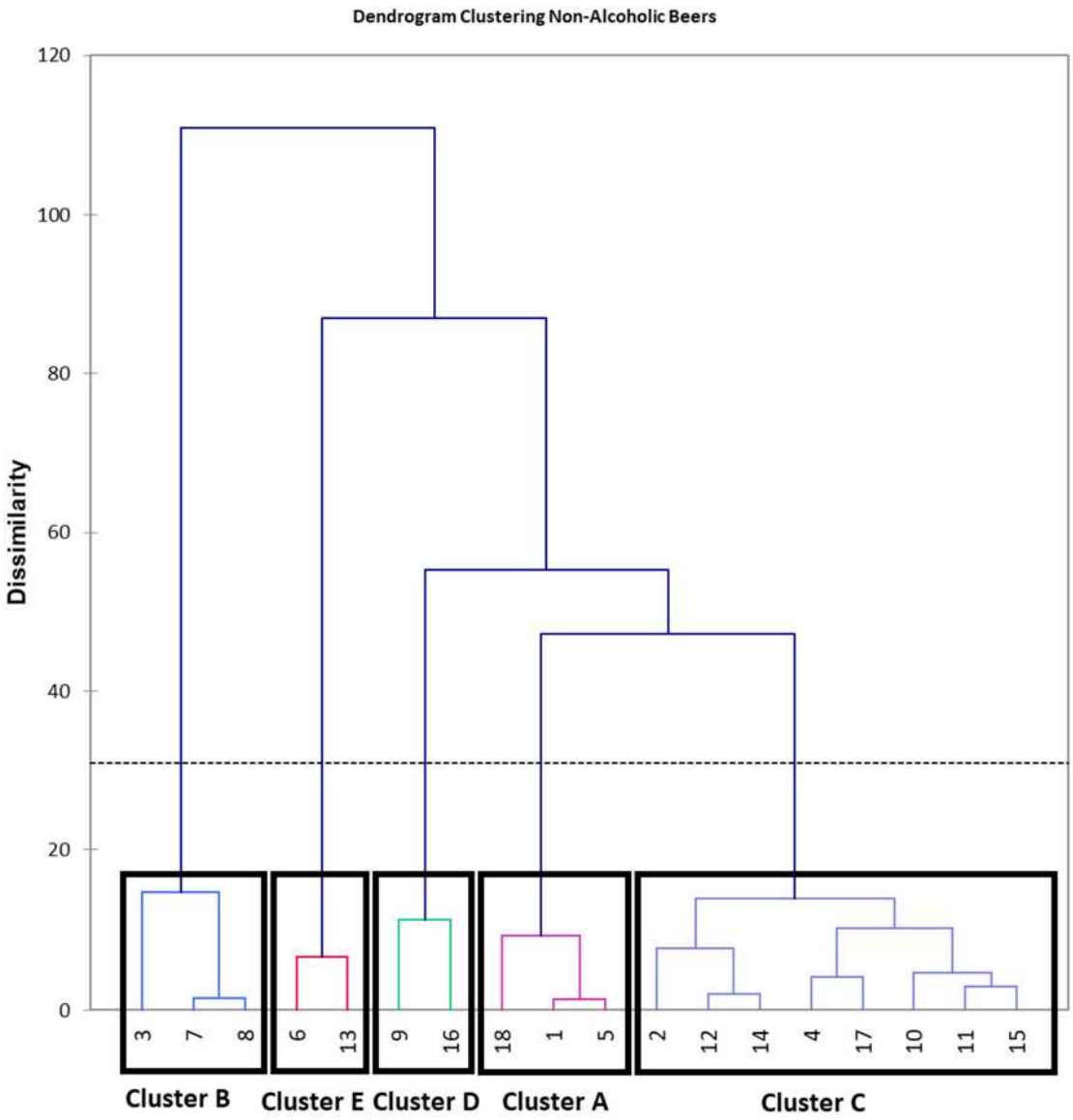
Appendix Table 6: *Attributes, definitions and reference standards used in QDA trained sensory panel for lager and stout (n=10). Attributes with * are those used only or lagers and those with ** for attributes used only with stouts*

	Attribute	Description	Reference
Aroma/Flavour	Fruity/Estery	Esters derived from fermentation. Flavours including: strawberry, raspberry, peach, apricot, pineapple, banana, peardrops, candy sticks	0.5-10mg isoamyl acetate/L beer (FlavorActiv™); 0.05-0.25mg ethyl hexanoate/L beer (FlavorActiv™)
	Alcoholic/Solvent	Ethanol and higher alcohols from fermentation. Flavours including: Ethanolic, vinous, warming, raw, higher alcohols	Potable alcohol 0-9% ABV in beer
	Fruity/Citrus	Citrus fruit character from hops. Flavours including : Grapefruit, lemon, lime, orange	Hop essential oils
	Hop**	Fresh, resinous, herbal, grassy, spicy	0.01-0.2mg spicy hop essential oils/L beer (FlavorActiv™)
	Floral/Fragrant*	Floral character from hops. Flavours including: Geraniol, floral, fragrant, elderflower, perfumed	0-100ug geraniol/L beer (FlavorActiv™)
	Spicy/Grassy Hop*	Grassy character from hops. Flavours including : Freshly cut grass, resinous, herbal, grassy, spicy	10-40ug eugenol/L beer (FlavorActiv™); Cis-3-Hexenol (FlavorActiv™)
	Cereal	Cereal character from grains. Flavours including: Cereal, grainy, hay, straw, worty, bran	10ug 2-methyl propiondaldehyde/L beer (FlavorActiv™)
	Malty	Malted cereal from grains. Flavours including: Malty, nutty, vanilla	Malt extract Vanilla exctract
	Caramel**	Caramel, nutty, fudge	3-ethyl,2,5-dimethylpyrazine (FlavorActiv™)
	Burnt**	Roasted, burnt, ashy	N/A
	DMS	Dimethyl sulphide (part of the sulphury character of lagers). Flavours including: Sweetcorn, baked beans, tinned tomatoes	10-150ug dimethyl sulphide /L beer (FlavorActiv™)
	Other Sulphur	Any other sulphurs found in lager beers. Flavours including: Sulphidic (eggy), sulphitic (struck match), yeasty, bready, meaty, drainy, garlic, onion, cooked veg., lightstruck.	5-125ug hydrogen sulphide/L beer (FlavorActiv™); 15mg sodium sulphite/L beer (FlavorActiv™);

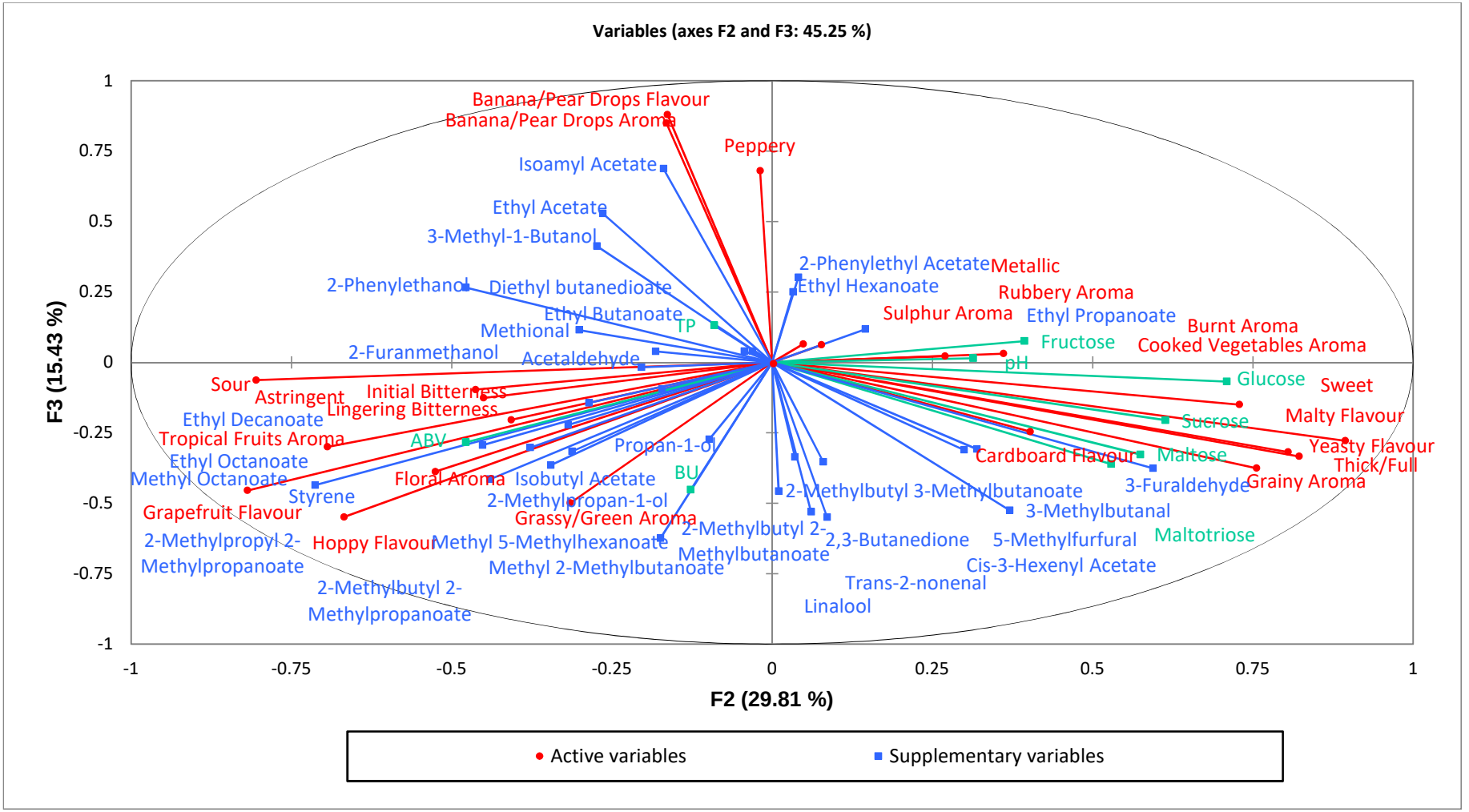
			0.05-0.3ug dimethyl trisulphide/L beer (FlavorActiv™); 2-15mg acetaldehyde/L beer (FlavorActiv™); 8-600ug diacetyl/L beer (FlavorActiv™); 30-200mg acetic acid/L beer (FlavorActiv™); chlorophenol (FlavorActiv™)
Taste	Sweet	Sweet taste from residual sugars. Flavours including: Sugar, saccharin, honey, syrup	Sucrose
	Sour	Sour taste. Acidic, mouthpuckering	90-300mg citric acid/L beer (FlavorActiv™)
	Bitter	Bitter taste mostly from hop alpha iso acids. Tonic water, quinine	5 Bitterness Units (BU) iso-α-acids/L beer (FlavorActiv™)
Mouthfeel	Astringent	Astringent mouthfeel, mainly from hop components. Tannic, drying, black tea	Saponin (FlavorActiv™)
	Body	Mouthfeel, density, associated to non fermentable sugars, ethanol and higher alcohols. Thick, viscous, full, thin, watery	N/A
	Linger	Length of flavour in mouth. Aftertaste, length, intensity	N/A



Appendix Figure 1: *Correlation plot summarising the relationship between saliva, ethanol and corresponding logP values across samples. Red denotes a strong positive relationship and blue denotes a strong negative relationship.*



Appendix Figure 2: *Dendogram of agglomerative hierarchical clustering (AHC) of non-alcoholic beer samples*



Appendix Figure 3: *Correlation biplot of all physicochemical, instrumental and sensory data showing significant attributes present on principal component 2 and 3. Attributes in red show QDA sensory attributes, those in green show instrumental analysis and those in blue show volatile compounds found through GC-MS*

References

ABInBev. (2018). *ABInBev: A Healthier World* [Online]. ABInBev. Available: https://ab-inbev.co.uk/social-responsibility/a-healthier-world/ [23.04.2018].

Ahuja, K. & Rawat, A. (2019). *Non-Alcoholic Beer Market Size By Product, Technology, Sales Channel, Low Alcohol By Technology and Sales Channel, Industry Analysis Report, Regional Outlook, Growth Potential, Price Trends, Competitive Market Share & Forecast, 2017 – 2024* [Online]. Global Market Insights. Available: https://www.gminsights.com/industry-analysis/non-alcoholic-beer-market [accessed 01.10.2019].

Alcantara, B. M., Marques, D. R., Chinellato, M. M., Marchi, L. B., da Costa, S. C. & Monteiro, A. R. G. (2016). Assessment of quality and production process of a non-alcoholic stout beer using reverse osmosis. *Journal of the Institute of Brewing,* 122**,** 714-718. https://doi.org/10.1002/jib.368.

Alexi, N., Nanou, E., Lazo, O., Guerrero, L., Grigorakis, K. & Byrne, D. V. (2018). Check-All-That-Apply (CATA) with semi-trained assessors: Sensory profiles closer to descriptive analysis or consumer elicited data? *Food Quality and Preference,* 64**,** 11-20. https://doi.org/10.1016/j.foodqual.2017.10.009.

Allen, A. L., McGeary, J. E. & Hayes, J. E. (2014). Polymorphisms in TRPV1 and TAS2Rs Associate with Sensations from Sampled Ethanol. *Alcoholism: Clinical and Experimental Research,* 38**,** 2550-2560. https://doi.org/10.1111/acer.12527.

Amerine, M. A. & Roessler, E. B. (1976). *Wines : their sensory evaluation,* San Francisco: W.H. Freeman & Co Ltd.

Ammari, A. & Schroen, K. (2019). Effect of Ethanol and Temperature on Partition Coefficients of Ethyl Acetate, Isoamyl Acetate, and Isoamyl Alcohol: Instrumental and Predictive Investigation. *Journal of Chemical & Engineering Data,* 64**,** 3224-3230. https://doi.org/10.1021/acs.jced.8b01125.

Analytica-EBC (2018). *European Brewery Convention (EBC). Lower Boiling Point Volatile Compounds in Beer by Gas Chromatography, 9.39,* BrewUp, Brussels, Belgium.

Andrés-Iglesias, C., García-Serna, J., Montero, O. & Blanco, C. A. (2015). Simulation and flavor compound analysis of dealcoholized beer via one-step vacuum distillation. *Food Research International,* 76**,** 751-760. http://dx.doi.org/10.1016/j.foodres.2015.07.017.

Aprea, E., Biasioli, F., Mark, T. D. & Gasperi, F. (2007). PTR-MS study of esters in water and water/ethanol solutions: Fragmentation patterns and partition coefficients. *International Journal of Mass Spectrometry,* 262**,** 114-121. https://doi.org/10.1016/j.ijms.2006.10.016.

Ares, G., Alcaire, F., Antúnez, L., Vidal, L., Giménez, A. & Castura, J. C. (2017). Identification of drivers of (dis)liking based on dynamic sensory profiles: Comparison of Temporal Dominance of Sensations and Temporal Check-all-that-apply. *Food Research International,* 92**,** 79-87. http://dx.doi.org/10.1016/j.foodres.2016.12.016.

Ares, G., Antúnez, L., Bruzzone, F., Vidal, L., Giménez, A., Pineau, B., Beresford, M. K., Jin, D., Paisley, A. G., Chheang, S. L., Roigard, C. M. & Jaeger, S. R. (2015). Comparison of sensory product profiles

generated by trained assessors and consumers using CATA questions: Four case studies with complex and/or similar samples. *Food Quality and Preference,* 45**,** 75-86. https://doi.org/10.1016/j.foodqual.2015.05.007.

Ares, G., Barreiro, C., Deliza, R., Gimenez, A. & Gambaro, A. (2010). Application of a check-all-that-apply question to the development of chocolate milk desserts. *Journal of Sensory Studies,* 25**,** 67-86. https://doi.org/10.1111/j.1745-459X.2010.00290.x.

Ares, G. & Jaeger, S. R. (2015). '11 - Check-all-that-apply (CATA) questions with consumers in practice: experimental considerations and impact on outcome'. in *Rapid Sensory Profiling Techniques*. Woodhead Publishing.

Arrieta, A. A., Rodriguez-Mendez, M. L., de Saja, J. A., Blanco, C. A. & Nimubona, D. (2010). Prediction of bitterness and alcoholic strength in beer using an electronic tongue. *Food Chemistry,* 123**,** 642-646. https://doi.org/10.1016/j.foodchem.2010.05.006.

ASBC Method of Analysis (2015). *American Society of Brewing Chemists. Total Polyphenols, Beer-35,* The Society: St Paul, MN, USA.

ASBC Method of Analysis (2018). *American Society of Brewing Chemists. Bitterness Units (International Method), Beer-25A,* The Society: St Paul, MN, USA.

Athès, V., Peña y Lillo, M., Bernard, C., Pérez-Correa, R. & Souchon, I. (2004). Comparison of Experimental Methods for Measuring Infinite Dilution Volatilities of Aroma Compounds in Water/Ethanol Mixtures. *Journal of Agricultural and Food Chemistry,* 52**,** 2021-2027. https://doi.org/10.1021/jf0350257.

Ayed, C., Martins, S., Williamson, A. M. & Guichard, E. (2018). Understanding fat, proteins and saliva impact on aroma release from flavoured ice creams. *Food Chemistry,* 267**,** 132-139. https://doi.org/10.1016/j.foodchem.2017.10.127.

Aznar, M., Tsachaki, M., Linforth, R. S. T., Ferreira, V. & Taylor, A. J. (2004). Headspace analysis of volatile organic compounds from ethanolic systems by direct APCI-MS. *International Journal of Mass Spectrometry,* 239**,** 17-25. http://dx.doi.org/10.1016/j.ijms.2004.09.001.

Bachmanov, A. A. & Beauchamp, G. K. (2007). Taste receptor genes. *Annual Review of Nutrition,* 27**,** 389-414. https://doi.org/10.1146/annurev.nutr.26.061505.111329.

Baker, A. K., Castura, J. C. & Ross, C. F. (2016). Temporal Check-All-That-Apply Characterization of Syrah Wine. *Journal of Food Science,* 81**,** 1521-1529. https://doi.org/10.1111/1750-3841.13328.

Basso, R. F., Alcarde, A. R. & Portugal, C. B. (2016). Could non-Saccharomyces yeasts contribute on innovative brewing fermentations? *Food Research International,* 86**,** 112-120. https://doi.org/10.1016/j.foodres.2016.06.002.

Bellut, K. & Arendt, E. K. (2019). Chance and Challenge: Non-Saccharomyces Yeasts in Nonalcoholic and Low Alcohol Beer Brewing – A Review. *Journal of the American Society of Brewing Chemists,* 77**,** 77-91. https://doi.org/10.1080/03610470.2019.1569452.

Bellut, K., Michel, M., Zarnkow, M., Hutzler, M., Jacob, F., De Schutter, D. P., Daenen, L., Lynch, K. M., Zannini, E. & Arendt, E. K. (2018). Application of Non-Saccharomyces Yeasts Isolated from Kombucha in the Production of Alcohol-Free Beer. *Fermentation,* 4**,** 66-85. https://doi.org/10.3390/fermentation4030066.

Ben-David, A., Bason, S., Jopp, J., Oren, Y. & Freger, V. (2006). Partitioning of organic solutes between water and polyamide layer of RO and NF

membranes: Correlation to rejection. *Journal of Membrane Science,* 281**,** 480-490. https://doi.org/10.1016/j.memsci.2006.04.017.
Blackmore, H., Hidrio, C., Godineau, P. & Yeomans, M. R. (2020). The effect of implicit and explicit extrinsic cues on hedonic and sensory expectations in the context of beer. *Food Quality and Preference,* 81**,** 17. https://doi.org/10.1016/j.foodqual.2019.103855.
Blake, R. S., Monks, P. S. & Ellis, A. M. (2009). Proton-Transfer Reaction Mass Spectrometry. *Chemical Reviews,* 109**,** 861-896. https://doi.org/10.1021/cr800364q.
Blanco, C. A., Andres-Iglesias, C. & Montero, O. (2016). Low-alcohol Beers: Flavor Compounds, Defects, and Improvement Strategies. *Critical Reviews in Food Science and Nutrition,* 56**,** 1379-1388. https://doi.org/10.1080/10408398.2012.733979.
Boehm, M. W., Yakubov, G. E., Delwiche, J. F., Stokes, J. R. & Baier, S. K. (2019). Enabling the Rational Design of Low-Fat Snack Foods: Insights from In Vitro Oral Processing. *Journal of Agricultural and Food Chemistry,* 67**,** 8725-8734. https://doi.org/10.1021/acs.jafc.9b02121.
Boehm, M. W., Yakubov, G. E., Stokes, J. R. & Baier, S. K. (2020). The role of saliva in oral processing: Reconsidering the breakdown path paradigm. *Journal of Texture Studies,* 51**,** 67-77. https://doi.org/10.1111/jtxs.12411.
Boelrijk, A. E. M., Basten, W., Burgering, M., Gruppen, H., Voragen, A. & Smit, G. (2003) The effect of co-solvent on the release of key flavours in alcoholic beverages; comparing in vivo with artificial mouth-MS Nose measurements. *Flavour Research at the Dawn of the Twenty-first Century : proceedings of the 10th Weurman flavour research symposium.* Paris, London.
Boothroyd, E. L., Linforth, R. S. T. & Cook, D. J. (2012). Effects of Ethanol and Long-Chain Ethyl Ester Concentrations on Volatile Partitioning in a Whisky Model System. *Journal of Agricultural and Food Chemistry,* 60**,** 9959-9966. https://doi.org/10.1021/jf3022892.
Brandts, J. F. & Hunt, L. (1967). Thermodynamics of protein denaturation. III. Denaturation of ribonuclease in water and in aqueous urea and aqueous ethanol mixtures. *Journal of the American Chemical Society,* 89**,** 4826-4838. https://doi.org/10.1021/ja00995a002.
Branyik, T., Silva, D. P., Baszczynski, M., Lehnert, R. & Silva, J. (2012). A review of methods of low alcohol and alcohol-free beer production. *Journal of Food Engineering,* 108**,** 493-506. https://doi.org/10.1016/j.jfoodeng.2011.09.020.
Breslin, P. A. S., Gilmore, M. M., Beauchamp, G. K. & Green, B. G. (1993). Psychophysical evidence that oral astringency is a tactile sensation. *Chemical Senses,* 18**,** 405-417. https://doi.org/10.1093/chemse/18.4.405.
Briggs, D. E., Boulton, C. A., Brookes, P. A. & Stevens, R. (2004). *Brewing Science and Practice,* Cambridge, England: Woodhead Publishing Limited.
Bruzzone, F., Ares, G. & Gimenez, A. (2012). Consumers' texture perception of milk desserts. II - Comparison with trained assessors' data. *Journal of Texture Studies,* 43**,** 214-226. https://doi.org/10.1111/j.1745-4603.2011.00332.x.
Buck, L. B. (2004). Olfactory receptors and odor coding in mammals. *Nutrition reviews,* 62**,** 184-188.
Burmeister, M. S., Drummond, C. J., Pfisterer, E. A., Hysert, D. W., Sin, Y. O., Sime, K. J. & Hawthorne, D. B. (1992). Measurement of Volatile Sulfur Compounds in Beer Using Gas Chromatography with a Sulfur

Chemiluminescence Detector. *Journal of the American Society of Brewing Chemists,* 50**,** 53-58. https://doi.org/10.1094/ASBCJ-50-0053.
Canonico, L., Agarbati, A., Comitini, F. & Ciani, M. (2016). Torulaspora delbrueckii in the brewing process: A new approach to enhance bioflavour and to reduce ethanol content. *Food Microbiology,* 56**,** 45-51. https://doi.org/10.1016/j.fm.2015.12.005.
Capece, A., Romaniello, R., Siesto, G. & Romano, P. (2018). Conventional and Non-Conventional Yeasts in Beer Production. *Fermentation,* 4**,** 38. https://doi.org/10.3390/fermentation4020038.
Carlsberg. (2019). *Zero Irresponsible Drinking* [Online]. Carlsberg. Available: https://www.carlsberggroup.com/sustainability/our-ambitions/zero-irresponsible-drinking/ [accessed 03.08.2020].
Castro, L. & Ross, C. (2013). The Effect of Protein and Carbohydrate Levels on the Chemical and Sensory Properties of Beer. *Journal of the American Society of Brewing Chemists,* 71**,** 186-192. https://doi.org/10.1094/ASBCJ-2013-0913-01.
Castura, J. C., Antúnez, L., Giménez, A. & Ares, G. (2016a). Temporal Check-All-That-Apply (TCATA): A novel dynamic method for characterizing products. *Food Quality and Preference,* 47**,** 79-90. http://dx.doi.org/10.1016/j.foodqual.2015.06.017.
Castura, J. C., Baker, A. K. & Ross, C. F. (2016b). Using contrails and animated sequences to visualize uncertainty in dynamic sensory profiles obtained from temporal check-all-that-apply (TCATA) data. *Food Quality and Preference,* 54**,** 90-100. https://doi.org/10.1016/j.foodqual.2016.06.011.
Catarino, M. (2010). *Production of Non-Alcoholic Beer with Reincorporation of Original Aroma Compounds.* PhD Thesis, University of Porto.
Catarino, M. & Mendes, A. (2011a). Dealcoholizing wine by membrane separation processes. *Innovative Food Science & Emerging Technologies,* 12**,** 330-337. https://doi.org/10.1016/j.ifset.2011.03.006.
Catarino, M. & Mendes, A. (2011b). Non-alcoholic beer—A new industrial process. *Separation and Purification Technology,* 79**,** 342-351. https://doi.org/10.1016/j.seppur.2011.03.020.
Catarino, M., Mendes, A., Madeira, L. & Ferreira, A. (2006). Beer dealcoholization by reverse osmosis. *Desalination,* 200**,** 397-399. https://doi.org/10.1016/j.desal.2006.03.346.
Catarino, M., Mendes, A., Madeira, L. M. & Ferreira, A. (2007). Alcohol Removal From Beer by Reverse Osmosis. *Separation Science and Technology,* 42**,** 3011-3027. https://doi.org/10.1080/01496390701560223.
Chaya, C., Pacoud, J., Ng, M., Fenton, A. & Hort, J. (2015). Measuring the emotional response to beer and the relative impact of sensory and packaging cues. *Journal of the American Society of Brewing Chemists,* 73**,** 49-60. https://doi.org/10.1094/ASBCJ-2015-0114-01.
Chrysochou, P. (2014). Drink to get drunk or stay healthy? Exploring consumers' perceptions, motives and preferences for light beer. *Food Quality and Preference,* 31**,** 156-163. http://dx.doi.org/10.1016/j.foodqual.2013.08.006.
Clapperton, J. F. (1974). Profile analysis and flavour discrimination in beer. *Journal of the Institute of Brewing,* 80**,** 164-173. https://doi.org/10.1002/j.2050-0416.1974.tb03599.x.
Clark, R., Hewson, L., Bealin-Kelly, F. & Hort, J. (2011a). The Interactions of CO2, Ethanol, Hop Acids and Sweetener on Flavour Perception in a Model Beer. *Chemosensory Perception,* 4**,** 42-54. https://doi.org/10.1007/s12078-011-9087-3.

Clark, R., Linforth, R., Bealin-Kelly, F. & Hort, J. (2011b). Effects of Ethanol, Carbonation and Hop Acids on Volatile Delivery in a Model Beer System. *Journal of the Institute of Brewing,* 117**,** 74-81. https://doi.org/10.1002/j.2050-0416.2011.tb00446.x.

Cliff, M. & Heymann, H. (1992). Descriptive analysis of oral pungency. *Journal of Sensory Studies,* 7**,** 279-290. https://doi.org/10.1111/j.1745-459X.1992.tb00195.x.

Comettomuniz, J. E., Garciamedina, M. R., Calvino, A. M. & Noriega, G. (1987). Interactions between CO2 oral pungency and taste. *Perception,* 16**,** 629-640. https://doi.org/10.1068/p160629.

Conner, J. M., Birkmyre, L., Paterson, A. & Piggott, J. R. (1998). Headspace concentrations of ethyl esters at different alcoholic strengths. *Journal of the Science of Food and Agriculture,* 77**,** 121-126. https://doi.org/10.1002/(SICI)1097-0010(199805)77:1%3C121::AID-JSFA14%3E3.0.CO;2-V.

Cowart, B. J. (1998). The addition of CO2 to traditional taste solutions alters taste quality. *Chem Senses,* 23**,** 397-402. https://doi.org/10.1093/chemse/23.4.397.

D'Angelo, M., Onori, G. & Santucci, A. (1994a). Self-association behaviour of alcohols in diluted aqueous solutions. *Il Nuovo Cimento D,* 16**,** 1499-1514. https://doi.org/10.1007/BF02462035.

D'Angelo, M., Onori, G. & Santucci, A. (1994b). Self-association of monohydric alcohols in water: Compressibility and infrared absorption measurements. *The Journal of Chemical Physics,* 100**,** 3107-3113. https://doi.org/10.1063/1.466452.

de Araujo, I. E. T., Rolls, E. T., Kringelbach, M. L., McGlone, F. & Phillips, N. (2003). Taste-olfactory convergence, and the representation of the pleasantness of flavour, in the human brain. *European Journal of Neuroscience,* 18**,** 2059-2068. https://doi.org/10.1046/j.1460-9568.2003.02915.x.

De Francesco, G., Sannino, C., Sileoni, V., Marconi, O., Filippucci, S., Tasselli, G. & Turchetti, B. (2018). Mrakia gelida in brewing process: An innovative production of low alcohol beer using a psychrophilic yeast strain. *Food Microbiology,* 76**,** 354-362. https://doi.org/10.1016/j.fm.2018.06.018.

De Francesco, G., Sileoni, V., Marconi, O. & Perretti, G. (2015a). Pilot Plant Production of Low-Alcohol Beer by Osmotic Distillation. *Journal of the American Society of Brewing Chemists,* 73**,** 41-48. https://doi.org/10.1094/asbcj-2015-0112-01.

De Francesco, G., Turchetti, B., Sileoni, V., Marconi, O. & Perretti, G. (2015b). Screening of new strains of Saccharomycodes ludwigii and Zygosaccharomyces rouxii to produce low-alcohol beer. *Journal of the Institute of Brewing,* 121**,** 113-121. https://doi.org/10.1002/jib.185.

De Keukeleire, D. (2000). Fundamentals of beer and hop chemistry. *Química Nova,* 23**,** 108-112. http://dx.doi.org/10.1590/S0100-40422000000100019

del Olmo, A., Blanco, C. A., Palacio, L., Pradanos, P. & Hernandez, A. (2014). Pervaporation methodology for improving alcohol-free beer quality through aroma recovery. *Journal of Food Engineering,* 133**,** 1-8. https://doi.org/10.1016/j.jfoodeng.2014.02.014.

del Olmo, A., Blanco, C. A., Palacio, L., Prádanos, P. & Hernández, A. (2012). Setting up of a Method of Pervaporation for Improving Alcohol-Free Beer. *Procedia Engineering,* 44**,** 1005-1006. https://doi.org/10.1016/j.proeng.2012.08.654.

Delarue, J. & Loescher, E. (2004). Dynamics of food preferences: a case study with chewing gums. *Food Quality and Preference,* 15**,** 771-779. http://dx.doi.org/10.1016/j.foodqual.2003.11.005.

Demiglio, P. & Pickering, G. (2008). The influence of ethanol and pH on the taste and mouthfeel sensations elicited by red wine. *Journal of food, agriculture & environment,* 6**,** 143-150.

Department of Health & Social Care. (2018a). *The Government response to the consultation on low alcohol descriptors* [Online]. GOV.UK: Crown Copyright. Available: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/763904/government-response-to-consultation-on-low-alcohol-descriptors.pdf [accessed 21.07.18].

Department of Health & Social Care. (2018b). *Low Alcohol Descriptors: A consultation on the use of low alcohol descriptors* [Online]. GOV.UK: Crown Copyright. Available: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/690516/low_alcohol_descriptors_consultation_document.pdf [accessed 21.07.18].

Desai, N. T., Shepard, L. & Drake, M. A. (2013). Sensory properties and drivers of liking for Greek yogurts. *Journal of Dairy Science,* 96**,** 7454-7466. https://doi.org/10.3168/jds.2013-6973.

Dessirier, J.-M., Simons, C. T., Carstens, M. I., O'Mahony, M. & Carstens, E. (2000). Psychophysical and Neurobiological Evidence that the Oral Sensation Elicited by Carbonated Water is of Chemogenic Origin. *Chemical Senses,* 25**,** 277-284. 10.1093/chemse/25.3.277.

Diban, N., Athes, V., Bes, M. & Souchon, I. (2008). Ethanol and aroma compounds transfer study for partial dealcoholization of wine using membrane contactor. *Journal of Membrane Science,* 311**,** 136-146. https://doi.org/10.1016/j.memsci.2007.12.004.

Dinu, V., Gadon, A., Hurst, K., Lim, M., Ayed, C., Gillis, R. B., Adams, G. G., Harding, S. E. & Fisk, I. D. (2019a). An enzymatically controlled mucoadhesive system for enhancing flavour during food oral processing. *npj Science of Food,* 3**,** 11. https://doi.org/10.1038/s41538-019-0043-y.

Dinu, V., Gillis, R. B., MacCalman, T., Lim, M., Adams, G. G., Harding, S. E. & Fisk, I. D. (2019b). Submaxillary Mucin: its Effect on Aroma Release from Acidic Drinks and New Insight into the Effect of Aroma Compounds on its Macromolecular Integrity. *Food Biophysics,* 14**,** 278-286. https://doi.org/10.1007/s11483-019-09574-2.

Dinu, V., Liu, C., Ali, J., Ayed, C., Gershkovich, P., Adams, G. G., Harding, S. E. & Fisk, I. D. (2018). Analytical ultracentrifugation in saliva research: Impact of green tea astringency and its significance on the in-vivo aroma release. *Scientific Reports,* 8**,** 13350. https://doi.org/10.1038/s41598-018-31625-w.

Dooley, L., Lee, Y.-s. & Meullenet, J.-F. (2010). The application of check-all-that-apply (CATA) consumer profiling to preference mapping of vanilla ice cream and its comparison to classical external preference mapping. *Food Quality and Preference,* 21**,** 394-401. https://doi.org/10.1016/j.foodqual.2009.10.002.

Dziondziak, K. & Holsten Brauerei, A. G. (1989). *Method for the production of low-alcohol or alcohol-free beer.* German patent application US4814188A.

Earthy, P. J., MacFie, H. J. H. & Hedderley, D. (1997). Effect of question order on sensory perception and preference in central location trials. *Journal*

of Sensory Studies, 12**,** 215-237. https://doi.org/10.1111/j.1745-459X.1997.tb00064.x.
Ejikeme, M. E., Lgbokwe, P. & Ejikeme, P. (2013). Dealcoholization of Beer using Osmotic Membrane Distillation. *The Pacific Journal of Science and Technology,* 14**,** 301-309.
Elmore, J. S. (2015). 'Aroma extract analysis'. in*:* Parker, J. K., Elmore, J. S. & Methven, L. (eds.) *Flavour Development, Analysis and Perception in Food and Beverages*. Cambridge, UK: Woodhead Publishing.
Elmore, S. & Langley, K. (1996). Novel Vessel for the Measurement of Dynamic Flavor Release in Real Time from Liquid Foods. *Journal of Agricultural and Food Chemistry,* 44**,** 3560-3563. https://doi.org/10.1021/jf950687k.
Escalona, H., Piggott, J. R., Conner, J. M. & Paterson, A. (1999). Effect of ethanol strength on the volatility of higher alcohols and aldehydes. *Italian Journal of Food Science,* 11**,** 241-248. https://doi.org/10.1016/S0167-4501(98)80081-2.
Estela-Escalante, W. D., Rosales-Mendoza, S., Moscosa-Santillán, M. & González-Ramírez, J. E. (2016). Evaluation of the fermentative potential of Candida zemplinina yeasts for craft beer fermentation. *Journal of the Institute of Brewing,* 122**,** 530-535. https://doi.org/10.1002/jib.354.
Euromonitor. (2017a). *Beer in Western Europe* [Online]. Euromonitor International. Available: https://www.portal.euromonitor.com/portal/analysis/tab# [accessed 20.05.18].
Euromonitor. (2017b). *Non/Low Alcohol Beer - Market Sizes - Historical/Forecast - Total Volume* [Online]. London: Euromonitor. Available: https://www.portal.euromonitor.com/portal/Analysis/Tab# [accessed 10.01.2019].
Euromonitor. (2017c). *Non/Low Alcohol Beer: Can it Live up to the Hype?* [Online]. London: Euromonitor. Available: https://www.portal.euromonitor.com/portal/analysis/tab# [10.01.2019].
Euromonitor. (2019a). *Beer in the United Kingdom* [Online]. London: Euromonitor. Available: https://www.portal.euromonitor.com/portal/analysis/tab [10.01.2019].
Euromonitor. (2019b). *Beer in World - Datagraphics* [Online]. London: Euromonitor. Available: https://www.portal.euromonitor.com/portal/StatisticsEvolution/index [accessed 10.01.2019].
Euromonitor. (2020). *World Market for Alcoholic Drinks* [Online]. London: Euromonitor. Available: https://www.portal.euromonitor.com/portal/analysis/tab [accessed 08.08.2020].
European Parliament and Council (2011). On the provision of food information to consumers. European Parliament and Council*,* Regulation (EU) No 1169/2011.
Falkenberg, A. (2014). *Removal of Alcohol from Beer Using Membrane Processes.* MSc Thesis, University of Copenhagen.
Feng, X. & Huang, R. Y. M. (1997). Liquid Separation by Membrane Pervaporation: A Review. *Industrial & Engineering Chemistry Research,* 36**,** 1048-1066. https://doi.org/10.1021/ie960189g.
Fischer, C., Fischer, U. & Jakob, L. (1996) Impact of matrix variables ethanol, sugar, glycerol, pH and temperature on the partition coefficients of aroma compounds in wine and their kinetics of volatilisation. *Proceedings for the 4th International Symposium on Cool Climate*

Viticulture and Enology. Rochester, NY: New York State Agricultural Experiment Station.
Fisk, I. D. (2015). 'Aroma release'. in*:* Parker, J. K., Elmore, J. S. & Methven, L. (eds.) *Flavour Development, Analysis and Perception in Food and Beverages*. Woodhead Publishing.
Fontoin, H., Saucier, C., Teissedre, P. L. & Glories, Y. (2008). Effect of pH, ethanol and acidity on astringency and bitterness of grape seed tannin oligomers in model wine solution. *Food Quality and Preference,* 19**,** 286-291. https://doi.org/10.1016/j.foodqual.2007.08.004.
Forster, A. & Gahr, A. (2011). Aromahopfen und die Herstellung von Bieren mit reduziertem Alkoholgehalt. *Brauwelt International,* 18**,** 562-566.
Friel, E. N. & Taylor, A. J. (2001). Effect of salivary components on volatile partitioning from solutions. *Journal of Agricultural and Food Chemistry,* 49**,** 3898-3905. https://doi.org/10.1021/jf010371e.
Gambuti, A., Rinaldi, A., Lisanti, M. T., Pessina, R. & Moio, L. (2011). Partial dealcoholisation of red wines by membrane contactor technique: influence on colour, phenolic compounds and saliva precipitation index. *European Food Research and Technology,* 233**,** 647-655. https://doi.org/10.1007/s00217-011-1553-2.
García de la Torre, J. & Harding, S. E. (2013). Hydrodynamic modelling of protein conformation in solution: ELLIPS and HYDRO. *Biophysical reviews,* 5**,** 195-206. https://doi.org/10.1007/s12551-013-0102-6.
Gawel, R., Schulkin, A., Smith, P. A., Espinase, D. & McRae, J. M. (2020). Effect of dissolved carbon dioxide on the sensory properties of still white and red wines. *Australian Journal of Grape and Wine Research,* 26**,** 172-179. https://doi.org/10.1111/ajgw.12429.
Gawel, R., Van Sluyter, S. & Waters, E. J. (2007). The effects of ethanol and glycerol on the body and other sensory characteristics of Riesling wines. *Australian Journal of Grape and Wine Research,* 13**,** 38-45. https://doi.org/10.1111/j.1755-0238.2007.tb00070.x.
Gellynck, X., Kühne, B., Van Bockstaele, F., Van de Walle, D. & Dewettinck, K. (2009). Consumer perception of bread quality. *Appetite,* 53**,** 16-23. http://dx.doi.org/10.1016/j.appet.2009.04.002.
Gernat, D. C., Brouwer, E. & Ottens, M. (2019). Aldehydes as Wort Off-Flavours in Alcohol-Free Beers—Origin and Control. *Food and Bioprocess Technology*. https://doi.org/10.1007/s11947-019-02374-z.
Giacalone, D., Ribeiro, L. M. & Frost, M. B. (2016). Perception and Description of Premium Beers by Panels with Different Degrees of Product Expertise. *Beverages,* 2**,** 11. https://doi.org/10.3390/beverages2010005.
Gil, M., Estevez, S., Kontoudakis, N., Fort, F., Canals, J. M. & Zamora, F. (2013). Influence of partial dealcoholization by reverse osmosis on red wine composition and sensory characteristics. *European Food Research and Technology,* 237**,** 481-488. https://doi.org/10.1007/s00217-013-2018-6.
Gittings, S., Turnbull, N., Henry, B., Roberts, C. J. & Gershkovich, P. (2015). Characterisation of human saliva as a platform for oral dissolution medium development. *European Journal of Pharmaceutics and Biopharmaceutics,* 91**,** 16-24. https://doi.org/10.1016/j.ejpb.2015.01.007.
Green, A. A. (1933). The Preparation of Acetate and Phosphate Buffer Solutions of Known pH and Ionic Strength. *Journal of the American Chemical Society,* 55**,** 2331-2336. https://doi.org/10.1021/ja01333a018.

Green, B. G. (1987). The Sensitivity of the Tongue to Ethanol. *Annals of the New York Academy of Sciences,* 510**,** 315-317. https://doi.org/10.1111/j.1749-6632.1987.tb43541.x.
Green, B. G. (1991). Capsaicin cross-desensitization on the tongue: psychophysical evidence that oral chemical irritation is mediated by more than one sensory pathway. *Chemical senses,* 16**,** 675-689.
Green, B. G. (1992). The effects of temperature and concentration on the perceived intensity and quality of carbonation. *Chemical Senses,* 17**,** 435-450. https://doi.org/10.1093/chemse/17.4.435.
Green, B. G. (1993). Oral astringency: A tactile component of flavor. *Acta Psychologica,* 84**,** 119-125. https://doi.org/10.1016/0001-6918(93)90078-6.
Guichard, E. (2002). Interactions between flavour compounds and food ingredients and their influence on flavour perception. *Food Reviews International,* 18**,** 49-70. https://doi.org/10.1081/FRI-120003417.
Guinard, J.-X., Pangborn, R. M. & Lewis, M. J. (1986). Effect of repeated ingestion on temporal perception of bitterness in beer. *Journal of the American Society of Brewing Chemists,* 44**,** 28-32. https://doi.org/10.1094/ASBCJ-44-0028.
Guinard, J.-X., Uotani, B. & Schlich, P. (2001). Internal and external mapping of preferences for commercial lager beers: comparison of hedonic ratings by consumers blind versus with knowledge of brand and price. *Food Quality and Preference,* 12**,** 243-255. http://dx.doi.org/10.1016/S0950-3293(01)00011-8.
Güzel, N., Güzel, M. & Savaş Bahçeci, K. (2020). 'Nonalcoholic Beer'. in*:* Galanakis, C. M. (eds.) *Trends in Non-alcoholic Beverages*. London, UK: Academic Press.
Heineken. (2019). *Responsible Consumption* [Online]. Heineken. Available: https://www.theheinekencompany.com/our-sustainability-story/our-strategy-and-achievements/responsible-consumption [accessed 03.08.2020].
Hellekant, G., Danilova, V., Roberts, T. & Ninomiya, Y. (1997). The taste of ethanol in a primate model: I. Chorda tympani nerve response in Macaca mulatta. *Alcohol,* 14**,** 473-484. http://dx.doi.org/10.1016/S0741-8329(96)00215-7.
Hewson, L., Hollowood, T., Chandra, S. & Hort, J. (2009). Gustatory, Olfactory and Trigeminal Interactions in a Model Carbonated Beverage. *Chemosensory Perception,* 2**,** 94-107. 10.1007/s12078-009-9043-7.
Higgins, M. J., Bakke, A. J. & Hayes, J. E. (2020). Personality traits and bitterness perception influence the liking and intake of pale ale style beers. *Food Quality and Preference,* 86**,** 103994. https://doi.org/10.1016/j.foodqual.2020.103994.
Higgins, M. J., Gipple, J. T. & Hayes, J. E. (2021). Common bitter stimuli show differences in their temporal profiles before and after swallowing. *Food Quality and Preference,* 87**,** 104041. https://doi.org/10.1016/j.foodqual.2020.104041.
Hodenberg, G. W. (1991). Production of alcohol free beers using reverse osmosis. *Brauwelt International,* 2**,** 145-149.
Holmes, C. P., Hense, W., Donnelly, D. & Cook, D. J. (2014). Impacts of steam injection technology on volatile formation and stripping during wort boiling *Technical Quarterly of the Master Brewers' Association of the Americas,* 51**,** 33-41. https://doi.org/10.1094/TQ-51-2-0514-01.
Hoopman, T., Birch, G., Serghat, S., Portmann, M.-O. & Mathlouthi, M. (1993). Solute-solvent interactions and the sweet taste of small carbohydrates. Part II: Sweetness intensity and persistence in ethanol-water mixtures.

Food Chemistry, 46**,** 147-153. https://doi.org/10.1016/0308-8146(93)90028-E.
Humphrey, S. P. & Williamson, R. T. (2001). A review of saliva: normal composition, flow, and function. *J Prosthet Dent,* 85**,** 162-9. https://doi.org/10.1067/mpr.2001.113778.
Ickes, C. M. & Cadwallader, K. R. (2017). Effects of Ethanol on Flavour Perception in Alcoholic Beverages. *Chemosensory Perception*. https://doi.org/10.1007/s12078-017-9238-2.
Ivanov, K., Petelkov, I., Shopska, V., Denkova, R., Gochev, V. & Kostov, G. (2016). Investigation of mashing regimes for low-alcohol beer production. *Journal of the Institute of Brewing,* 122**,** 508-516. https://doi.org/10.1002/jib.351.
Jaeger, S. R., Beresford, M. K., Hunter, D. C., Alcaire, F., Castura, J. C. & Ares, G. (2017). Does a familiarization step influence results from a TCATA task? *Food Quality and Preference,* 55**,** 91-97. http://dx.doi.org/10.1016/j.foodqual.2016.09.001.
Jaeger, S. R., Chheang, S. L., Yin, J., Bava, C. M., Gimenez, A., Vidal, L. & Ares, G. (2013a). Check-all-that-apply (CATA) responses elicited by consumers: Within-assessor reproducibility and stability of sensory product characterizations. *Food Quality and Preference,* 30**,** 56-67. http://dx.doi.org/10.1016/j.foodqual.2013.04.009.
Jaeger, S. R., Giacalone, D., Roigard, C. M., Pineau, B., Vidal, L., Giménez, A., Frøst, M. B. & Ares, G. (2013b). Investigation of bias of hedonic scores when co-eliciting product attribute information using CATA questions. *Food Quality and Preference,* 30**,** 242-249. https://doi.org/10.1016/j.foodqual.2013.06.001.
Jamieson, L. & Wantling, E. (2017). 'Multiple-Ingestion Time–Intensity'. in*:* Hort, J., Kemp, S. E. & Hollowood, T. A. (eds.) *Time-Dependent Measures of Perception in Sensory Evaluation*. Chichester, UK: John Wiley & Sons, Ltd.
Jardim, C. D., de Souza, D., Machado, I. C. K., Pinto, L. M. N., Ramos, R. C. D. & Garavaglia, J. (2018). Sensory Profile, Consumer Preference and Chemical Composition of Craft Beers from Brazil. *Beverages,* 4**,** 12. https://doi.org/10.3390/beverages4040106.
Jiang, Z., Yang, B., Liu, X., Zhang, S., Shan, J., Liu, J. & Wang, X. (2017). A novel approach for the production of a non-alcohol beer (≤0.5% abv) by a combination of limited fermentation and vacuum distillation. *Journal of the Institute of Brewing,* 123**,** 533-536. https://doi.org/10.1002/jib.465.
Jones, P. R., Gawel, R., Francis, I. L. & Waters, E. J. (2008). The influence of interactions between major white wine components on the aroma, flavour and texture of model white wine. *Food Quality and Preference,* 19**,** 596-607. https://doi.org/10.1016/j.foodqual.2008.03.005.
Jouenne, E. & Crouzet, J. (2000). Effect of pH on Retention of Aroma Compounds by β-Lactoglobulin. *Journal of Agricultural and Food Chemistry,* 48**,** 1273-1277. https://doi.org/10.1021/jf990215w.
Kaipainen, A. (1992). A study of the aroma profiles of non-alcohol beer by thermal desorption and GC-MS. *Journal of High Resolution Chromatography,* 15**,** 751-755. https://doi.org/10.1002/jhrc.1240151110.
Kaneda, H., Kobayashi, N., Watari, J., Shinotsuka, K., Takashio, M. & Okahata, Y. (2002). A new taste sensor for evaluation of beer body and smoothness using a lipid-coated quartz crystal microbalance. *Journal of the American Society of Brewing Chemists,* 60**,** 71-76. https://doi.org/10.1094/ASBCJ-60-0071.

Kappes, S. M., Schmidt, S. J. & Lee, S.-Y. (2006). Color Halo/Horns and Halo-Attribute Dumping Effects within Descriptive Analysis of Carbonated Beverages. *Journal of Food Science,* 71**,** 590-595. https://doi.org/10.1111/j.1750-3841.2006.00161.x.

Kavanagh, T. E., Clarke, B. J., Gee, P. S., Miles, M. & Nicholson, B. N. (1991). Volatile Flavour Compounds in Low Alcohol Beers. *Tech. Q. Master Brew. Assoc. Am,* 28**,** 111-118.

Kemp, S., Hollowood, T. & Hort, J. (2009). *Sensory Evaluation : A Practical Handbook,* Chichester, UK: John Wiley & Sons.

Kemp, S. E., Hort, J. & Hollowood, T. (2017). *Time-Dependent Measures of Perception in Sensory Evaluation,* Chichester, UK: John Wiley & Sons.

Kemp, S. E., Hort, J. & Hollowood, T. (2018). *Descriptive analysis in sensory evaluation,* Chichester, UK: John Wiley & Sons.

Kielhorn, S. & Thorngate Iii, J. H. (1999). Oral sensations associated with the flavan-3-ols (+)-catechin and (-)-epicatechin. *Food Quality and Preference,* 10**,** 109-116. https://doi.org/10.1016/s0950-3293(98)00049-4.

King, E. S., Dunn, R. L. & Heymann, H. (2013). The influence of alcohol on the sensory perception of red wines. *Food Quality and Preference,* 28**,** 235-243. https://doi.org/10.1016/j.foodqual.2012.08.013.

Kostas, E. T., White, D. A., Du, C. & Cook, D. J. (2016). Selection of yeast strains for bioethanol production from UK seaweeds. *Journal of Applied Phycology,* 28**,** 1427-1441. https://doi.org/10.1007/s10811-015-0633-2.

Krebs, G., Müller, M., Becker, T. & Gastl, M. (2018). Characterization of the macromolecular and sensory profile of non-alcoholic beers produced with various methods. *Food Research International*. https://doi.org/10.1016/j.foodres.2018.08.067.

Labanda, J., Vichi, S., Llorens, J. & López-Tamames, E. (2009). Membrane separation technology for the reduction of alcoholic degree of a white model wine. *LWT - Food Science and Technology,* 42**,** 1390-1395. https://doi.org/10.1016/j.lwt.2009.03.008.

Lachenmeier, D. W. (2014). Alcoholic beverage strength discrimination by taste may have an upper threshold. *Alcoholism, Clinical And Experimental Research,* 38**,** 2460. https://doi.org/10.1111/acer.12511.

Lafontaine, S., Senn, K., Dennenlöhr, J., Schubert, C., Knoke, L., Maxminer, J., Cantu, A., Rettberg, N. & Heymann, H. (2020). Characterizing Volatile and Nonvolatile Factors Influencing Flavor and American Consumer Preference toward Nonalcoholic Beer. *ACS Omega,* 5**,** 23308-23321. https://doi.org/10.1021/acsomega.0c03168.

Langstaff, S. A., Guinard, J. X. & Lewis, M. J. (1991). Instrumental evaluation of the mouthfeel of beer and correlation with sensory evaluation. *Journal of the Institute of Brewing,* 97**,** 427-433. https://doi.org/10.1002/j.2050-0416.1991.tb01081.x.

Langstaff, S. A. & Lewis, M. J. (1993). The mouthfeel of beer - a review. *Journal of the Institute of Brewing,* 99**,** 31-37. https://doi.org/10.1002/j.2050-0416.1993.tb01143.x.

Lawless, H. T. (2010). *Sensory evaluation of food : principles and practices,* London: Springer.

Lawson, K. W. & Lloyd, D. R. (1997). Membrane distillation. *Journal of Membrane Science,* 124**,** 1-25. https://doi.org/10.1016/S0376-7388(96)00236-0.

Lee, W. E. & Pangborn, R. M. (1986). Time intensity - the temporal aspects of sensory perception. *Food Technology,* 40**,** 71-82.

Lentz, M. (2018). The Impact of Simple Phenolic Compounds on Beer Aroma and Flavor. *Fermentation,* 4**,** 20. https://doi.org/10.3390/fermentation4010020.
Leskošek, I., Mitrović, M. & Nedović, V. (1995). Factors influencing alcohol and extract separation in beer dialysis. *World Journal of Microbiology & Biotechnology,* 11**,** 512-514. https://doi.org/10.1007/BF00286364.
Leskošek, I. J. & Mitrović, M. (1994). Optimisation of beer dialysis with cuprophane membranes. *Journal of the Institute of Brewing,* 100**,** 287-292. https://doi,org/10.1002/j.2050-0416.1994.tb00826.x.
Light, W., Mooney, L., Chu, H. & Wood, S. Alcohol removal from beer by reverse osmosis. AIChE Symposium Series, 1986. 1-8.
Liguori, L., De Francesco, G., Albanese, D., Mincione, A., Perretti, G., Di Matteo, M. & Russo, P. (2018a). Impact of Osmotic Distillation on the Sensory Properties and Quality of Low Alcohol Beer. *Journal of Food Quality,* 2018**,** 11. https://doi.org/10.1155/2018/8780725.
Liguori, L., De Francesco, G., Russo, P., Perretti, G., Albanese, D. & Di Matteo, M. (2015). Production and characterization of alcohol-free beer by membrane process. *Food and Bioproducts Processing,* 94**,** 158-168. https://doi.org/10.1016/j.fbp.2015.03.003.
Liguori, L., De Francesco, G., Russo, P., Perretti, G., Albanese, D. & Di Matteo, M. (2016). Quality Attributes of Low-Alcohol Top-Fermented Beers Produced by Membrane Contactor. *Food and Bioprocess Technology,* 9**,** 191-200. https://doi.org/10.1007/s11947-015-1612-y.
Liguori, L., Russo, P., Albanese, D. & Di Matteo, M. (2013a). Effect of process parameters on partial dealcoholization of wine by osmotic distillation. *Food and Bioprocess Technology,* 6**,** 2514-2524. https://doi.org/10.1007/s11947-012-0856-z.
Liguori, L., Russo, P., Albanese, D. & Di Matteo, M. (2013b). Evolution of quality parameters during red wine dealcoholization by osmotic distillation. *Food chemistry,* 140**,** 68-75. https://doi.org/10.1016/j.foodchem.2013.02.059.
Liguori, L., Russo, P., Albanese, D. & Di Matteo, M. (2018b). 'Production of Low-Alcohol Beverages: Current Status and Perspectives'. in*:* Grumezescu, A. M. & Holban, A. M. (eds.) *Food Processing for Increased Quality and Consumption*. London, UK: Academic Press.
Linforth, R., Martin, F., Carey, M., Davidson, J. & Taylor, A. J. (2002). Retronasal Transport of Aroma Compounds. *Journal of Agricultural and Food Chemistry,* 50**,** 1111-1117. https://doi.org/10.1021/jf011022n.
Lisanti, M. T., Gambuti, A., Genovese, A., Piombino, P. & Moio, L. (2013). Partial Dealcoholization of Red Wines by Membrane Contactor Technique: Effect on Sensory Characteristics and Volatile Composition. *Food and Bioprocess Technology,* 6**,** 2289-2305. https://doi.org/10.1007/s11947-012-0942-2.
Liu, S.-Q. & Quek, A. Y. H. (2016). Evaluation of Beer Fermentation with a Novel Yeast Williopsis saturnus. *Food Technology and Biotechnology,* 54**,** 403-412. https://doi.org/10.17113/ftb.54.04.16.4440.
Liu, Y., Li, H. & Du, J. (2011). Non-alcoholic Beer Production by Saccharomycodes Ludwigii. *Chinese Institute of Food Science and Technology,* 32**,** 186-190.
Löbs, A.-K., Schwartz, C. & Wheeldon, I. (2017). Genome and metabolic engineering in non-conventional yeasts: Current advances and applications. *Synthetic and Systems Biotechnology,* 2**,** 198-207. https://doi.org/10.1016/j.synbio.2017.08.002.
Long, J. S. (1997). *Regression models for categorical and limited dependent variables,* London, UK: Sage Publications.

Lopez, M., Alvarez, S., Riera, F. A. & Alvarez, R. (2002). Production of low alcohol content apple cider by reverse osmosis. *Industrial & Engineering Chemistry Research,* 41**,** 6600-6606. https://doi.org/10.1021/ie020155a.

Lyly, M., Salmenkallio-Marttila, M., Suortti, T., Autio, K., Poutanen, K. & Lähteenmäki, L. (2003). Influence of Oat β-Glucan Preparations on the Perception of Mouthfeel and on Rheological Properties in Beverage Prototypes. *Cereal Chemistry,* 80**,** 536-541. https://doi.org.10.1094/cchem.2003.80.5.536.

Malfliet, S., Goiris, K., Aerts, G. & De Cooman, L. (2009). Analytical-Sensory Determination of Potential Flavour Deficiencies of Light Beers. *Journal of the Institute of Brewing,* 115**,** 49-63. https://doi.org/10.1002/j.2050-0416.2009.tb00344.x.

Mandel, A. L., Peyrot des Gachons, C., Plank, K. L., Alarcon, S. & Breslin, P. A. S. (2010). Individual differences in AMY1 gene copy number, salivary α-amylase levels, and the perception of oral starch. *PloS one,* 5**,** e13352-e13352. https://doi.org/10.1371/journal.pone.0013352.

Mangindaan, D., Khoiruddin, K. & Wenten, I. G. (2018). Beverage dealcoholization processes: Past, present, and future. *Trends in Food Science & Technology,* 71**,** 36-45. https://doi.org/10.1016/j.tifs.2017.10.018.

Martin, S. & Pangborn, R. M. (1970). Taste interaction of ethyl alcohol with sweet, salty, sour and bitter compounds. *Journal of the Science of Food and Agriculture,* 21**,** 653-655. https://doi.org/10.1002/jsfa.2740211213.

Mattes, R. D. & DiMeglio, D. (2001). Ethanol perception and ingestion. *Physiology & Behavior,* 72**,** 217-229. https://doi.org/10.1016/s0031-9384(00)00397-8.

McMahon, K. M., Culver, C., Castura, J. C. & Ross, C. F. (2017). Perception of carbonation in sparkling wines using descriptive analysis (DA) and temporal check-all-that-apply (TCATA). *Food Quality and Preference,* 59**,** 14-26. https://doi.org/10.1016/j.foodqual.2017.01.017.

Meilgaard, M. C., Dalgliesh, C. E. & Clapperton, J. F. (1979). Beer Flavour Terminology. *Journal of the Institute of Brewing,* 85**,** 38-42. https://doi.org/10.1002/j.2050-0416.1979.tb06826.x.

Meillon, S., Urbano, C. & Schlich, P. (2009). Contribution of the Temporal Dominance of Sensations (TDS) method to the sensory description of subtle differences in partially dealcoholized red wines. *Food Quality and Preference,* 20**,** 490-499. https://doi.org/10.1016/j.foodqual.2009.04.006.

Meillon, S., Viala, D., Medel, M., Urbano, C., Guillot, G. & Schlich, P. (2010). Impact of partial alcohol reduction in Syrah wine on perceived complexity and temporality of sensations and link with preference. *Food Quality and Preference,* 21**,** 732-740. https://doi.org/10.1016/j.foodqual.2010.06.005.

Mello, L. S. S., Almeida, E. L. & Melo, L. (2019). Discrimination of sensory attributes by trained assessors and consumers in semi-sweet hard dough biscuits and their drivers of liking and disliking. *Food Research International,* 122**,** 599-609. https://doi.org/10.1016/j.foodres.2019.01.031.

Meyners, M. & Castura, J. C. (2016). Randomization of CATA attributes: Should attribute lists be allocated to assessors or to samples? *Food Quality and Preference,* 48**,** 210-215. https://doi.org/10.1016/j.foodqual.2015.09.014.

Meyners, M., Castura, J. C. & Carr, B. T. (2013). Existing and new approaches for the analysis of CATA data. *Food Quality and Preference,* 30**,** 309-319. https://doi.org/10.1016/j.foodqual.2013.06.010.
Michel, M., Kopecká, J., Meier-Dörnberg, T., Zarnkow, M., Jacob, F. & Hutzler, M. (2016). Screening for new brewing yeasts in the non-Saccharomyces sector with Torulaspora delbrueckii as model. *Yeast,* 33**,** 129-144. https://doi.org/10.1002/yea.3146.
Mintel. (2014). *Lightweight lager? Europeans embrace the non-alcoholic beer boom* [Online]. London: Mintel. Available: https://www.mintel.com/press-centre/food-and-drink/lightweight-lager-europeans-embrace-the-non-alcoholic-beer-boom [accessed 10.08.2017].
Mintel. (2015). *Attitudes towards Low- and Non-alcoholic Drink - UK - April 2015* [Online]. London: Mintel. Available: http://academic.mintel.com/display/736868/ [accessed 10.08.2017].
Mintel. (2017a). *Attitudes towards Low- and Non-alcoholic Drink - UK - February 2017* [Online]. London: Mintel. Available: http://academic.mintel.com/display/818025/ [accessed on 20.05.18].
Mintel. (2017b). *Beer - UK - December 2017* [Online]. London: Mintel. Available: http://academic.mintel.com/display/873535/ [accessed 07.01.18].
Mintel. (2018). *Alcoholic Drinks Review - UK - Feburary 2018* [Online]. London: Mintel. Available: http://academic.mintel.com/display/882573/ [accessed 03.04.18].
Mintel. (2019a). *Alcoholic Drinks Review - UK - February 2019* [Online]. Available: http://academic.mintel.com/display/920384/ [
Mintel. (2019b). *Attitudes towards Low- and Non-alcoholic Drink - UK - August 2019* [Online]. London: Mintel. Available: https://reports.mintel.com/display/919650/?fromSearch=%3Ffreetext%3Dnon-alcoholic [accessed on 12.03.19].
Mintel. (2019c). *Beer - UK - December 2019* [Online]. Available: https://reports.mintel.com/display/921450/?fromSearch=%3Ffreetext%3DBeer%2520UK [accessed on 12.03.20].
Missbach, B., Majchrzak, D., Sulzner, R., Wansink, B., Reichel, M. & Koenig, J. (2017). Exploring the flavor life cycle of beers with varying alcohol content. *Food Science & Nutrition,* 5**,** 889-895. https://doi.org/10.1002/fsn3.472.
Mitropoulou, A., Hatzidimitriou, E. & Paraskevopoulou, A. (2011). Aroma release of a model wine solution as influenced by the presence of non-volatile components. Effect of commercial tannin extracts, polysaccharides and artificial saliva. *Food Research International,* 44**,** 1561-1570. https://doi.org/10.1016/j.foodres.2011.04.023.
Molson Coors. (2016). *Our 2025 Imprint Goals* [Online]. Molson Coors. Available: https://www.molsoncoors.com/sustainability/our-imprint-goals [accessed 03.08.2020].
Montanari, L., Marconi, O., Mayer, H. & Fantozzi, P. (2009). 'Production of Alcohol-Free Beer'. in*:* Preedy, V. R. (eds.) *Beer in Health and Disease Prevention*. San Diego, USA: Academic Press.
Mortazavian, A. M., Razavi, S. H., Mousavi, S. M., Malganji, S. & Sohrabvandi, S. (2014). The effect of Saccharomyces strain and fermentation conditions on quality prameters of non-alcoholic beer. *J. Paramed. Sci,* 5**,** 21-26. https://doi.org/10.22037/jps.v5i2.5942.
Müller, M., Bellut, K., Tippmann, J. & Becker, T. (2017). Physical Methods for Dealcoholization of Beverage Matrices and their Impact on Quality

Attributes. *ChemBioEng Reviews,* 4**,** 310-326. https://doi.org/10.1002/cben.201700010.
Muller, R. (1990). The production of low-alcohol and alcohol-free beers by limited fermentations. *Ferment,* 3**,** 224-230.
Mundy, A. P. (1991). The determination of dimethyl sulphide in beer by headspace gas chromatography - a collaborative investigation of precision. *Journal of the Institute of Brewing,* 97**,** 45-46. https://doi.org/10.1002/j.2050-0416.1991.tb01052.x.
Muñoz-González, C., Feron, G., Guichard, E., Rodríguez-Bencomo, J. J., Martín-Álvarez, P. J., Moreno-Arribas, M. V. & Pozo-Bayón, M. Á. (2014a). Understanding the Role of Saliva in Aroma Release from Wine by Using Static and Dynamic Headspace Conditions. *Journal of Agricultural and Food Chemistry,* 62**,** 8274-8288. https://doi.org/10.1021/jf503503b.
Muñoz-González, C., Pérez-Jiménez, M., Criado, C. & Pozo-Bayón, M. Á. (2019). Effects of Ethanol Concentration on Oral Aroma Release After Wine Consumption. *Molecules,* 24**,** 3253-3263. https://doi.org/10.3390/molecules24183253.
Muñoz-González, C., Rodríguez-Bencomo, J. J., Moreno-Arribas, M. V. & Pozo-Bayón, M. Á. (2014b). Feasibility and application of a retronasal aroma-trapping device to study in vivo aroma release during the consumption of model wine-derived beverages. *Food Science & Nutrition,* 2**,** 361-370. https://doi.org/10.1002/fsn3.111.
Narziß, L., Miedaner, H., Kern, E. & Leibhard, M. (1992). Technology and composition of non-alcoholic beers. Processes using arrested fermentation. *Brauwelt International,* 4**,** 396-410.
Nevoigt, E., Pilger, R., Mast-Gerlach, E., Schmidt, U., Freihammer, S., Eschenbrenner, M., Garbe, L. & Stahl, U. (2002). Genetic engineering of brewing yeast to reduce the content of ethanol in beer. *FEMS Yeast Research,* 2**,** 225-232. https://doi.org/10.1016/S1567-1356(02)00076-4.
Nikolaidis, A. & Moschakis, T. (2018). On the reversibility of ethanol-induced whey protein denaturation. *Food Hydrocolloids,* 84**,** 389-395. https://doi.org/10.1016/j.foodhyd.2018.05.051.
Nolden, A. A. & Hayes, J. E. (2015). Perceptual Qualities of Ethanol Depend on Concentration, and Variation in These Percepts Associates with Drinking Frequency. *Chemosensory perception,* 8**,** 149-157. https://doi.org/10.1007/s12078-015-9196-5.
Nurgel, C. & Pickering, G. (2005). Contribution of glycerol, ethanol and sugar to the perception of viscosity and density elicited by model white wines. *Journal of Texture Studies,* 36**,** 303-323. https://doi.org/10.1111/j.1745-4603.2005.00018.x.
Obreque-Slíer, E., Peña-Neira, Á. & López-Solís, R. (2010). Enhancement of Both Salivary Protein−Enological Tannin Interactions and Astringency Perception by Ethanol. *Journal of Agricultural and Food Chemistry,* 58**,** 3729-3735. https://doi.org/10.1021/jf903659t.
Office For National Statistics. (2017). *Statistical bulletin: Alcohol specific deaths in the UK: registered in 2016* [Online]. Office for National Statistics. Available: https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/causesofdeath/bulletins/alcoholrelateddeathsintheunitedkingdom/registeredin2016 [accessed 18.04.2018].
Oladokun, O., Tarrega, A., James, S., Smart, K., Hort, J. & Cook, D. (2016). The impact of hop bitter acid and polyphenol profiles on the perceived

bitterness of beer. *Food Chemistry,* 205**,** 212-220. http://dx.doi.org/10.1016/j.foodchem.2016.03.023.
Pagès-Hélary, S., Andriot, I., Guichard, E. & Canon, F. (2014). Retention effect of human saliva on aroma release and respective contribution of salivary mucin and α-amylase. *Food Research International,* 64**,** 424-431. https://doi.org/10.1016/j.foodres.2014.07.013.
Peltz, M. & Shellhammer, T. (2017). Ethanol Content Has Little Effect on the Sensory Orthonasal Detection Threshold of Hop Compounds in Beer. *Journal of the American Society of Brewing Chemists,* 75**,** 221-227. https://doi.org/10.1094/ASBCJ-2017-3994-01.
Perpete, P. & Collin, S. (1999). Contribution of 3-methylthiopropionaldehyde to the worty flavor of alcohol-free beers. *Journal of Agricultural and Food Chemistry,* 47**,** 2374-2378. https://doi.org/10.1021/jf9811323.
Perpete, P. & Collin, S. (2000). Influence of beer ethanol content on the wort flavour perception. *Food Chemistry,* 71**,** 379-385. https://doi.org/10.1016/S0308-8146(00)00179-5.
Petkovska, M., Leskošek, I. & Nedović, V. (1997). Analysis of Mass Transfer in Beer Diafiltration with Cellulose-Based and Polysulfone Membranes. *Food and Bioproducts Processing,* 75**,** 247-252. https://doi.org/10.1205/096030897531658.
Philippe, E., Seuvre, A. M., Colas, B., Langendorff, V., Schippa, C. & Voilley, A. (2003). Behavior of flavor compounds in model food systems: a thermodynamic study. *Journal of Agricultural and Food Chemistry,* 51**,** 1393-1398. https://doi.org/10.1021/jf020862e.
Pickering, G. J., Heatherbell, D. A., Vanhanen, L. P. & Barnes, M. F. (1998). The effect of ethanol concentration on the temporal perception of viscosity and density in white wine. *American Journal of Enology and Viticulture,* 49**,** 306-318.
Pilipovik, M. V. & Riverol, C. (2005). Assessing dealcoholization systems based on reverse osmosis. *Journal of Food Engineering,* 69**,** 437-441. https://doi.org/10.1016/j.jfoodeng.2004.08.035.
Pineau, N., de Bouillé, A. G., Lepage, M., Lenfant, F., Schlich, P., Martin, N. & Rytz, A. (2012). Temporal Dominance of Sensations: What is a good attribute list? *Food Quality and Preference,* 26**,** 159-165. https://doi.org/10.1016/j.foodqual.2012.04.004.
Pineau, N., Schlich, P., Cordelle, S., Mathonnière, C., Issanchou, S., Imbert, A., Rogeaux, M., Etiévant, P. & Köster, E. (2009). Temporal Dominance of Sensations: Construction of the TDS curves and comparison with time–intensity. *Food Quality and Preference,* 20**,** 450-455. https://doi.org/10.1016/j.foodqual.2009.04.005.
Piornos, J. A., Delgado, A., de La Burgade, R. C. J., Methven, L., Balagiannis, D. P., Koussissi, E., Brouwer, E. & Parker, J. K. (2019). Orthonasal and retronasal detection thresholds of 26 aroma compounds in a model alcohol-free beer: Effect of threshold calculation method. *Food Research International,* 123**,** 317-326. https://doi.org/10.1016/j.foodres.2019.04.034.
Ployon, S., Morzel, M. & Canon, F. (2017). The role of saliva in aroma release and perception. *Food Chemistry,* 226**,** 212-220. https://doi.org/10.1016/j.foodchem.2017.01.055.
Pollock, D. (1990). Technischal University Munich.
Popper, R., Rosenstock, W., Schraidt, M. & Kroll, B. J. (2004). The effect of attribute questions on overall liking ratings. *Food Quality and Preference,* 15**,** 853-858. https://doi.org/10.1016/j.foodqual.2003.12.004.

Porretta, S. & Donadini, G. (2008). A Preference Study for No Alcohol Beer in Italy Using Quantitative Concept Analysis. *Journal of the Institute of Brewing,* 114**,** 315-321. 10.1002/j.2050-0416.2008.tb00775.x.

Preedy, V. R. (2011). *Beer in Health and Disease Prevention,* London, UK: Elsevier Science.

Prescott, J., Soo, J., Campbell, H. & Robells, C. (2004). Responses of PROP taster groups to variations in sensory qualities within foods and beverages. *Physiology & Behavior,* 82**,** 459-469. https://doi.org/10.1016/j.physbeh.2004.04.009.

Ramsey, I., Ross, C., Ford, R., Fisk, I., Yang, Q., Gomez-Lopez, J. & Hort, J. (2018). Using a combined temporal approach to evaluate the influence of ethanol concentration on liking and sensory attributes of lager beer. *Food Quality and Preference,* 68**,** 292-303. https://doi.org/10.1016/j.foodqual.2018.03.019.

Rehm, J., Lachenmeier, D. W., Llopis, E. J., Imtiaz, S. & Anderson, P. (2016). Evidence of reducing ethanol content in beverages to reduce harmful use of alcohol. *The Lancet Gastroenterology & Hepatology,* 1**,** 78-83. https://doi.org/10.1016/S2468-1253(16)30013-9.

Riu-Aumatell, M., Miro, P., Serra-Cayuela, A., Buxaderas, S. & Lopez-Tamames, E. (2014). Assessment of the aroma profiles of low-alcohol beers using HS-SPME-GC-MS. *Food Research International,* 57**,** 196-202. https://doi.org/10.1016/j.foodres.2014.01.016.

Rogers, L. (2017). *Discrimination Testing in Sensory Science: A Practical Handbook,* Cambridge, UK: Woodhead Publishings.

Rolls, E. T., Verhagen, J. V. & Kadohisa, M. (2003). Representations of the texture of food in the primate orbitofrontal cortex: Neurons responding to viscosity, grittiness, and capsaicin. *Journal of Neurophysiology,* 90**,** 3711-3724. https://doi.org/10.1152/jn.00515.2003.

Roper, S. D. (2007). Signal transduction and information processing in mammalian taste buds. *Pflugers Arch,* 454**,** 759-76. https://doi.org/10.1007/s00424-007-0247-x.

Russo, P., Liguori, L., Albanese, D., Crescitelli, A. & Di Matteo, M. (2013). Investigation of osmotic distillation technique for beer dealcoholization. *Chemical Engineering,* 32**,** 1735-1740. https://www.aidic.it/cet/13/32/290.pdf.

Saerens, S. & Hendrik Swiegers, J. (2014). *Production of low-alcohol or alcohol-free beer with pichia kluyveri yeast strains*. Denmark patent application WO2014135673A2.

Saison, D., De Schutter, D. P., Uyttenhove, B., Delvaux, F. & Delvaux, F. R. (2009). Contribution of staling compounds to the aged flavour of lager beer by studying their flavour thresholds. *Food Chemistry,* 114**,** 1206-1215. https://doi.org/10.1016/j.foodchem.2008.10.078.

Scheraga, H. A. & Mandelkern, L. (1953). Consideration of the Hydrodynamic Properties of Proteins1,2. *Journal of the American Chemical Society,* 75**,** 179-184. https://doi.org/10.1021/ja01097a047.

Schmelzle, A., Lindemann, B. & Methner, F.-J. (2013). Sensory descriptive analysis and consumer acceptance of non-alcoholic beer. *BrewingScience,* 66**,** 144-153.

Schur, F. & Sauer, P. (1990). *Process for the preparation of beer with low alcohol content*. Switzerland patent application 4971807.

Schutte, C. F. (2003). The rejection of specific organic compounds by reverse osmosis membranes. *Desalination,* 158**,** 285-294. https://doi.org/10.1016/S0011-9164(03)00466-1.

Scinska, A., Koros, E., Habrat, B., Kukwa, A., Kostowski, W. & Bienkowski, P. (2000). Bitter and sweet components of ethanol taste in humans. *Drug*

and Alcohol Dependence, 60**,** 199-206. https://doi.org/10.1016/s0376-8716(99)00149-0.
SeekingAlpha. (2016). *The Kings of Beers is Becoming a Non-Beer Company* [Online]. Available: https://seekingalpha.com/article/3991710-king-beers-becoming-non-beer-company [accessed 22.06.17].
Segal, D. S. & Stockwell, T. (2009). Low alcohol alternatives: A promising strategy for reducing alcohol related harm. *International Journal of Drug Policy,* 20**,** 183-187. https://doi.org/10.1016/j.drugpo.2008.06.001.
Selecky, R. & Smogrovicova, D. (2007). Technological and microbiological aspects of low-alcoholic beer production. *Chemicke Listy,* 101**,** 542-549.
Silva, A. P., Jager, G., van Bommel, R., van Zyl, H., Voss, H.-P., Hogg, T., Pintado, M. & de Graaf, C. (2016). Functional or emotional? How Dutch and Portuguese conceptualise beer, wine and non-alcoholic beer consumption. *Food Quality and Preference,* 49**,** 54-65. http://dx.doi.org/10.1016/j.foodqual.2015.11.007.
Silva, A. P., Jager, G., Voss, H.-P., van Zyl, H., Hogg, T., Pintado, M. & de Graaf, C. (2017). What's in a name? The effect of congruent and incongruent product names on liking and emotions when consuming beer or non-alcoholic beer in a bar. *Food Quality and Preference,* 55**,** 58-66. http://dx.doi.org/10.1016/j.foodqual.2016.08.008.
Simons, C. T., Dessirier, J.-M., Carstens, M. I., O'Mahony, M. & Carstens, E. (1999). Neurobiological and Psychophysical Mechanisms Underlying the Oral Sensation Produced by Carbonated Water. *The Journal of Neuroscience,* 19**,** 8134-8144.
Small, D. M. & Prescott, J. (2005). Odor/taste integration and the perception of flavor. *Experimental Brain Research,* 166**,** 345-357. https://doi.org/10.1007/s00221-005-2376-9.
Sohrabvandi, S., Mousavi, S. M., Razavi, S. H., Mortazavian, A. M. & Rezaei, K. (2010). Alcohol-free Beer: Methods of Production, Sensorial Defects, and Healthful Effects. *Food Reviews International,* 26**,** 335-352. https://doi.org/10.1080/87559129.2010.496022.
Solomon, O. F. & Ciută, I. Z. (1962). Détermination de la viscosité intrinsèque de solutions de polymères par une simple détermination de la viscosité. *Journal of Applied Polymer Science,* 6**,** 683-686. https://doi.org/10.1002/app.1962.070062414.
Stein, W. (1993). Dealcoholisation of Beer. *MBAA - Technical Quarterly,* 30**,** 54-57.
Stone, H., Bleibaum, R. N. & Thomas, H. A. (2012a). 'Descriptive Analysis'. in*:* Stone, H., Bleibaum, R. N. & Thomas, H. A. (eds.) *Sensory Evaluation Practices (Fourth Edition)*. San Diego: Academic Press.
Stone, H., Bleibaum, R. N. & Thomas, H. A. (2012b). 'Introduction to Sensory Evaluation'. in*:* Stone, H., Bleibaum, R. N. & Thomas, H. A. (eds.) *Sensory Evaluation Practices (Fourth Edition)*. San Diego: Academic Press.
Stone, H. & Sidel, J. L. (2004). 'Descriptive Analysis'. in*:* Stone, H. & Sidel, J. L. (eds.) *Sensory Evaluation Practices (Fourth Edition)*. San Diego: Academic Press.
Stovicek, V., Holkenbrink, C. & Borodina, I. (2017). CRISPR/Cas system for yeast genome engineering: advances and applications. *FEMS yeast research,* 17**,** 1-16. https://doi.org/10.1093/femsyr/fox030.
Sudre, J., Pineau, N., Loret, C. & Martin, N. (2012). Comparison of methods to monitor liking of food during consumption. *Food Quality and*

Preference, 24**,** 179-189. https://doi.org/10.1016/j.foodqual.2011.10.013.
Symoneaux, R., Le Quéré, J.-M., Baron, A., Bauduin, R. & Chollet, S. (2015). Impact of CO2 and its interaction with the matrix components on sensory perception in model cider. *LWT-Food Science and Technology,* 63**,** 886-891. https://doi.org/10.1016/j.lwt.2015.04.037.
Taylor, A. J. (2002). Release and Transport of Flavors In Vivo: Physicochemical, Physiological, and Perceptual Considerations. *Comprehensive Reviews in Food Science and Food Safety,* 1**,** 45-57. https://doi.org/10.1111/j.1541-4337.2002.tb00006.x.
Taylor, A. J., Linforth, R. S. T., Harvey, B. A. & Blake, A. (2000). Atmospheric pressure chemical ionisation mass spectrometry for in vivo analysis of volatile flavour release. *Food Chemistry,* 71**,** 327-338. https://doi.org/10.1016/S0308-8146(00)00182-5.
Taylor, A. J. & Roberts, D. D. (2004). *Flavor Perception,* Oxford, UK: Blackwell Publishing.
Taylor, A. J., Tsachaki, M., Lopez, R., Morris, C., Ferreira, V. & Wolf, B. (2010). 'Odorant Release from Alcoholic Beverages'. in*:* Da Costa, N. C. & Cannon, R. J. (eds.) *Flavors in Noncarbonated Beverages*. Washington, USA: American Chemical Society.
Taylor, D. (1990). The importance of pH control during brewing. *Technical quarterly-Master Brewers Association of the Americas (USA),* 27**,** 131-136.
Taylor, D. E. & Pangborn, R. M. (1990). Temporal aspects of hedonic responses. *Journal of Sensory Studies,* 4**,** 241-247. https://doi.org/10.1111/j.1745-459X.1990.tb00475.x.
Thomas, A., Chambault, M., Dreyfuss, L., Gilbert, C. C., Hegyi, A., Henneberg, S., Knippertz, A., Kostyra, E., Kremer, S., Silva, A. P. & Schlich, P. (2017). Measuring temporal liking simultaneously to Temporal Dominance of Sensations in several intakes. An application to Gouda cheeses in 6 Europeans countries. *Food Research International,* 99**,** 426-434. https://doi.org/10.1016/j.foodres.2017.05.035.
Thomas, A., Visalli, M., Cordelle, S. & Schlich, P. (2015). Temporal Drivers of Liking. *Food Quality and Preference,* 40**,** 365-375. https://doi.org/10.1016/j.foodqual.2014.03.003.
Trevisani, M., Smart, D., Gunthorpe, M. J., Tognetto, M., Barbieri, M., Campi, B., Amadesi, S., Gray, J., Jerman, J. C. & Brough, S. J. (2002). Ethanol elicits and potentiates nociceptor responses via the vanilloid receptor-1. *Nature neuroscience,* 5**,** 546-551. https://doi.org/10.1038/nn0602-852.
Tsachaki, M. (2006). *Factors determining aroma release from wine-based systems.* PhD Thesis, University of Nottingham.
Tsachaki, M., Gady, A. L., Kalopesas, M., Linforth, R. S. T., Athes, V., Marin, M. & Taylor, A. J. (2008). Effect of ethanol, temperature, and gas flow rate on volatile release from aqueous solutions under dynamic headspace dilution conditions. *Journal of Agricultural and Food Chemistry,* 56**,** 5308-5315. https://doi.org/10.1021/jf800225y.
Tsachaki, M., Linforth, R. S. T. & Taylor, A. J. (2005). Dynamic headspace analysis of the release of volatile organic compounds from ethanolic systems by direct APCI-MS. *Journal of Agricultural and Food Chemistry,* 53**,** 8328-8333. https://doi.org/10.1021/jf051202n.
Upadhyaya, J., Pydi, S. P., Singh, N., Aluko, R. E. & Chelikani, P. (2010). Bitter taste receptor T2R1 is activated by dipeptides and tripeptides.

Biochem Biophys Res Commun, 398**,** 331-5. https://doi.org/10.1016/j.bbrc.2010.06.097.
Van Dieren, B. (1995) Yeast metabolism and the production of alcohol free beer. *European Brewery Convention Monograph XXIV, EBC.* Espoo, Finland.
van Koningsveld, G. A., Gruppen, H., de Jongh, H. H. J., Wijngaards, G., van Boekel, M. A. J. S., Walstra, P. & Voragen, A. G. J. (2002). Effects of Ethanol on Structure and Solubility of Potato Proteins and the Effects of Its Presence during the Preparation of a Protein Isolate. *Journal of Agricultural and Food Chemistry,* 50**,** 2947-2956. https://doi.org/10.1021/jf011202x.
van Ruth, S. M., Grossmann, I., Geary, M. & Delahunty, C. M. (2001). Interactions between artificial saliva and 20 aroma compounds in water and oil model systems. *Journal of Agricultural and Food Chemistry,* 49**,** 2409-2413. https://doi.org/10.1021/jf001510f.
Varavuth, S., Jiraratananon, R. & Atchariyawut, S. (2009). Experimental study on dealcoholization of wine by osmotic distillation process. *Separation and Purification Technology,* 66**,** 313-321. https://doi.org/10.1016/j.seppur.2008.12.011.
Varela, P. & Ares, G. (2014). *Novel techniques in sensory characterization and consumer profiling,* London, UK: CRC Press.
Vázquez-Araújo, L., Parker, D. & Woods, E. (2013). Comparison of Temporal–Sensory Methods for Beer Flavor Evaluation. *Journal of Sensory Studies,* 28**,** 387-395. https://doi.org/10.1111/joss.12064.
Veldhuizen, M. G., Wuister, M. J. P. & Kroeze, J. H. A. (2006). Temporal aspects of hedonic and intensity responses. *Food Quality and Preference,* 17**,** 489-496. http://dx.doi.org/10.1016/j.foodqual.2005.07.004.
Verbelen, P. J., De Schutter, D. P., Delvaux, F., Verstrepen, K. J. & Delvaux, F. R. (2006). Immobilized yeast cell systems for continuous fermentation applications. *Biotechnology Letters,* 28**,** 1515-1525. https://doi.org/10.1007/s10529-006-9132-5.
Verma, V. & Rawat, A. (2018). *Non-Alcoholic Beer Market Size By Product, Technology, Sales Channel, Low Alcohol By Technology and Sales Channel, Industry Analysis Report, Regional Outlook, Growth Potential, Price Trends, Competitive Market Share & Forecast, 2017 – 2024* [Online]. Global Market Insights. Available: https://www.gminsights.com/industry-analysis/non-alcoholic-beer-market [accessed 01.10.18].
Vidal, S., Courcoux, P., Francis, L., Kwiatkowski, M., Gawel, R., Williams, P., Waters, E. & Cheynier, V. (2004). Use of an experimental design approach for evaluation of key wine components on mouth-feel perception. *Food Quality and Preference,* 15**,** 209-217. https://doi.org/10.1016/S0950-3293(03)00059-4.
Wenten, I. G., Dharmawijaya, P. T., Aryanti, P. T. P., Mukti, R. R. & Khoiruddin (2017). LTA zeolite membranes: current progress and challenges in pervaporation. *RSC Advances,* 7**,** 29520-29539. https://doi.org/.1039/C7RA03341A.
Wenten, I. G. & Khoiruddin (2016). Reverse osmosis applications: Prospect and challenges. *Desalination,* 391**,** 112-125. https://doi.org/10.1016/j.desal.2015.12.011.
Williams, A. (1972). Flavour effects of ethanol in alcoholic beverages. *Flavour Ind,* 3**,** 604-607.
Williams, A. A. & Rosser, P. R. (1981). Aroma enhancing effects of ethanol. *Chemical Senses,* 6**,** 149-153. 10.1093/chemse/6.2.149.

World Health Organisation. (2018). *Global Status Report on Alcohol and Health 2018* [Online]. Geneva, Switzerland: WHO. Available: https://www.who.int/substance_abuse/publications/global_alcohol_report/en/ [

Wu, G., Ross, C. F., Morris, C. F. & Murphy, K. M. (2017). Lexicon Development, Consumer Acceptance, and Drivers of Liking of Quinoa Varieties. *Journal of Food Science,* 82**,** 993-1005. https://doi.org/10.1111/1750-3841.13677.

Yakubov, G., Gibbins, H., Proctor, G. & Carpenter, G. (2014). Oral Mucosa: Physiological and Physicochemical Aspects. *Mucoadhesive Materials and Drug Delivery Systems***,** 1-38. https://doi.org/10.1002/9781118794203.ch01.

Yakubov, G. E. (2014). 'Saliva Lubrication'. in*:* Ligtenberg, A. & Veerman, E. (eds.) *Saliva: Secretion and Functions*. Amsterdam, The Netherlands: Karger.

Yang, N., Liu, C., Liu, X., Degn, T. K., Munchow, M. & Fisk, I. (2016). Determination of volatile marker compounds of common coffee roast defects. *Food Chemistry,* 211**,** 206-214. https://doi.org/10.1016/j.foodchem.2016.04.124.

Yang, Q., Kraft, M., Shen, Y., MacFie, H. & Ford, R. (2019). Sweet Liking Status and PROP Taster Status impact emotional response to sweetened beverage. *Food Quality and Preference,* 75**,** 133-144. https://doi.org/10.1016/j.foodqual.2019.02.016.

Zufall, C. & Wackerbauer, K. (2000a). The dealcoholisation of beer by dialysis - influencing beer quality by process engineering. *Monatsschrift Fur Brauwissenschaft,* 53**,** 164-179.

Zufall, C. & Wackerbauer, K. (2000b). Process engineering parameters for the dealcoholisation of beer by means of falling film evaporation and its influence on beer quality. *Monatsschrift Fur Brauwissenschaft,* 53**,** 124-137.