Tribo-rheology as an investigative tool for the elucidation of mouthfeel properties in alcoholic and non-alcoholic beer

by

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A thesis submitted to the University of Birmingham for the degree of

Doctor of Philosophy

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December 2023

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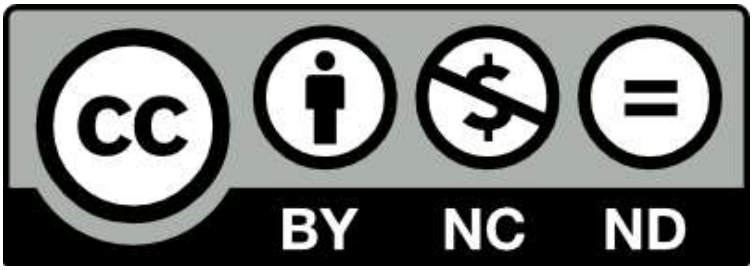

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Abstract

Interest in low and no-alcohol beers has markedly increased in recent years, both from consumers and scientifically. Despite the increasing breadth of published work on the area little has been directed towards mouthfeel properties. This attribute is well known to be challenging to assess, normally requiring tasting panels with significant training and experience to produce useable data. While tasting panels are used in other food and beverage areas, tribology and tribo-rheology have been utilized for other examples, offering an instrumental mechanism to assess lubrication properties of a product or system. This work aims to investigate the feasibility of utilizing tribometry based techniques in the assessment of properties of both experimentally relevant systems and commercially relevant products, to validate the methodology for this system as well as investigate the molecular causes of the effects observed.

Initial work was focused around selecting a system that was reproducible and could provide the sensitivity required for analysis of closely related samples are low axial forces and speeds relevant to oral processing. It was found that Tribo-rheology offered such a solution and proved able to differentiate between closely related commercial beer products as well as demonstrate differences between aqueous solutions with very similar compositions. Using this methodology behavioural differences were identified between several commercial beers both non-alcoholic and alcoholic, produced by the same brewery, conversely some products were shown to closely match their alcohol containing versions. The method was also used to investigate potential causes for differences, in ethanol itself, sugar (maltose), polysaccharide (maltodextrin) and sodium chloride in water. This identified some unexpected patterns,

whereby low concentrations of molecules would cause higher friction than pure water, indicating that products low in specific molecules may face worse lubricity outcomes than those where the component is entirely absent. This was of particular note for ethanol, where concentrations vary commercially and previously any amount of alcohol was largely considered to be positive for friction and thus mouthfeel.

Having demonstrated the techniques capability to differentiate between finished products, a microstructure approach was undertaken to attempt to elucidate the possible causes of the effects previously measured. This approach is to the authors knowledge novel, particularly in the scope of different molecules examined as well as their mixtures with others. This data indicated that even similar classes of molecules, e.g., inorganic chloride salts, can exhibit markedly different behaviour individually but more importantly when mixed with other molecules. These interactions were not easily predictable based on any property of the molecule, indicating that the interactions in lubrication are complex and varied. It was also found that many molecules exhibit unexpectedly strong effects despite concentrations below 100 parts per million, this included strong disruptive capabilities for acetoin and isoamyl alcohol. Volatile organic molecules are not classically considered to be of importance in tribology and when examined alone in water the effects were very minimal, once mixed with others however, there was a significant change in the tribological properties when compared to either molecule alone. This presented the possibility that molecules below taste thresholds could be causing changes in mouthfeel properties of products without presenting a clear taste profile, which would cause their identification to be challenging.

Following the microstructure-based study, the idea of manipulating the content of a beer post fermentation to achieve a desired goal was examined, this used proteins and amino acids, which had been demonstrated to have strong effects on friction in water previously. The analysis was conducted in water, a defined model beer analogue, a commercial 0% ABV beer and a commercial 4% ABV beer. Due to the exogenous nature of the nitrogen sources added here “real” concentrations were not used, a fixed molar concentration, allowed for more stoichiometrically fair comparison between all molecules, especially given the greatly different molecular masses. This methodology produced results demonstrating that while in water most amino acids provide positive lubricity this was not easily applicable to even a minimal defined beer analogue, while in commercial beer the results indicated that additions of amino acids or proteins could actively disrupt the existing lubricity, increasing friction as was seen with low concentrations of molecule in chapter 3. It was however seen that the proteins especially were able to entirely replace the previous systems lubrication properties, with 1.5 mM BSA in low alcohol beer showing no significant differences from 1.5 mM BSA in an alcoholic beer, despite there being clear difference between the two products before any additions were made.

Overall, it was found that tribo-rheology represents a useful tool in the investigation of lubricity in beer and beer related studies, able to demonstrate small differences between low concentration solutions and assist with elucidating the interactions potentially causing the observed effects. This method was then able to investigate the effect of additions of protein and amino acids to commercial and experimental beer products in terms of the potential effects on mouthfeel.

Acknowledgements

With the completion of this thesis, I would like to thank those who assisted me, as well as those who supported me prior to and during its completion.

I would like to thank my supervisors, Dr Tom Mills and Professor Ian Norton, for the opportunity to undertake this work, as well as their help and guidance throughout the project.

I want to thank Dr Mike Fry for his inspirational teaching when I first joined university and throughout my time at Reading, which led me to undertake this project.

I want to thank the members of the microstructure group, both students and staff throughout the time I was a part of it for supporting each other and helping us through what was a challenging period.

I want to thank Dr Michael-Alex Kamlow particularly for his assistance with the 3D printing used in this work, as well as his continued availability for beer related field work.

I would also like to thank Chandana for putting up with me during the final write up and for always being there for me as we build a life together, as well as for her support even when things have not been going as planned.

Finally, I would like to thank my family, especially my mother for her support in finding the position originally and her assistance in the review of papers and her unwavering support of my choices. I would also like to thank my grandmothers for their love and care throughout the years.

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Table 6: Simplified friction changes table for proteinogenic amino acids in water, values of 0 indicate that greater than 50% of points within that region were not significantly different from water ($p<0.05$ for significance), a value of 1 indicates that greater than 50% of points within that region significantly increased coefficient of friction compared to pure water, while a value of -1 indicates that greater than 50% of points within that region significantly reduced coefficient of friction compared to pure water – P163.

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List of abbreviations and initialisations

CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
ABV	Alcohol By Volume
ppm	Parts per million
BSA	Bovine Serum Albumin
N	Newton
PDMS	Polydimethylsiloxane
HPLC	High Performance Liquid Chromatography
DE	Dextrose Equivalent
PES	Poly-ethersulfone
EtOH	Ethanol
ACS	American Chemical Society
EtOAc	Ethyl acetate
ISA	Isoamyl alcohol
Da	Dalton
FDC	Food Data Central
TPSA	Topographic Polar Surface Area
2-PE	2-Phenylethanol
OG	Original Gravity
FG	Final Gravity
FTIR	Fourier Transform Infrared Spectroscopy

SEM	Scanning Electron Microscopy
UK	United Kingdom
HMRC	His Majesty Revenue and Customs
BCE	Before Common Era
mBar	Millibar
m/s	Meters per second
PVPP	Polyvinylpolypyrrolidone
DPN	Diphosphopyridine nucleotide
UHPLC	Ultra High Performance Liquid Chromatography
DCPIP	Dichlorophenol Indophenol
VOC	Volatile organic compound
SPME	Solid Phase Microextraction
PFBHA·HCl	(O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride
HSSE	Headspace Sorptive Extraction
SBSE	Stir Bar Sorptive Extraction
CBB	Coomassie brilliant Blue Binding
BCA	Bicinchoninic Acid
PAGE	Polyacrylamide Gel Electrophoresis
AAS	Atomic absorption spectroscopy
ICP-MS	Inductively coupled plasma-mass spectrometry
IPA0	India Pale ale 0% alcohol
IPA5	India Pale ale 5% alcohol

AA0	Amber ale 0% alcohol
AA5	Amber ale 4.3% alcohol
LA0	Lager 0% alcohol
LA46	Lager 4.6% alcohol
LA05	Lager 0.5% alcohol
PA05	Pale ale 0.5% alcohol
PA43	Pale ale 4.3% alcohol
MS05	Milk stout 0.5% alcohol
MS43	Milk stout 4.3% alcohol
GWB53	German wheat beer 5.3% alcohol
GWB0	German wheat beer 0% alcohol
CPA05	Citrus pale ale 0.5% alcohol
CPA45	Citrus pale ale 4.5% alcohol

Chapter 1- Introduction

1.0 Introduction

1.1 Context and Motivation

Excessive consumption of alcohol has long been known to cause serious physical and social health issues, in response to growing concerns, many alcoholic beverage companies began to produce lower or entirely non-alcoholic beverages. Initially this was focused almost entirely around beer, although cider and wine followed with non-alcoholic distilled products only recently becoming available to mainstream consumers. Early non-alcoholic beers were widely criticised by consumers for a variety of issues, largely centring on taste and or texture, due to this poor initial reception non-alcoholic products remained a small niche and many breweries elected not to advertise or produce an example. However more recently most large national/multi-national brewers have elected to produce low and or no-alcohol beer products for mass commercial adoption. This has been driven partly by demand from consumers and regulators but also from technological developments in dealcoholisation technology which promise improved products (Krebs et al. 2019).

These methods both new and historic can be broadly separated into two major groups, alcohol removed and low alcohol brewing. Initially it was accepted that the low alcohol products would initially be full strength beers which had the alcohol removed, this can be achieved by a wide range of methods, but these again are classified into two overarching technologies, thermal and membrane. Modern thermal methods are commonly used to produce commercially available beers (Liguori et al. 2015), these more recent versions utilize vacuum distillation, frequently also featuring a centrifugal mechanism to produce films for dealcoholizing (Ramsey et al. 2021) which enables a less destructive separation of ethanol from other constituent molecules owing to lower thermal stress, thus lowering oxidation

(Harrison 1970). Membrane technologies show a wider range of methodology, although the basis for them is largely similar, revolving around a membrane of varying composition which can selectively remove alcohol while leaving other molecules in the product (Alonso González and Parga-Dans 2020).

With the range of modern dealcoholisation technologies, various studies have been undertaken to assess the outcomes of the various methods on a product (Schur and Sauer 1990; Bellut and Arendt 2019; Popek and Halagarda 2017). These studies primarily focused on the more challenging to preserve molecules from the original beer namely the volatile organic aroma molecules. Due to the similarity in structure exhibited by smaller esters to ethanol, any process which is able to remove one is likely to have knock on effects for the other. The extensive body of work and well standardized methods utilized for the chemical analysis of beer contrast to the very limited range of work investigating the physical properties in terms of instrumental assessment of mouthfeel. While the use of tasting panels and expert assessors is common (Bellido-Milla et al. 2000) it presents a challenge, particularly in research brewing, where products may not be approved for human consumption immediately especially if they are produced from non-standard yeast and or are dealcoholized in experimental systems.

Low/no alcohol brewing represents an option for reduced mechanical complexity in production as well as presenting a low intervention methodology, which has proved to be popular with consumers within wine products (Klopper et al. 1986). This area holds significant promise for improvement of current methods reducing both energy and space usage for low alcohol brewing applications. A range of approaches have been undertaken to

achieve a desirable alcohol level, these take several main forms, limited fermentation is arguably the simplest in theory, where by fermentation is halted prematurely or attenuated by some other method, commonly temperature and or pH (Otter and Taylor 1967). Alternatively and arguably more promisingly non-standard yeast strains can be utilized, this may result in truly alcohol free products (Charry-Parra et al. 2011), but can cause regulatory issues around biological safety of relatively unknown organisms as well as often producing unrecognisable outputs to consumers expecting a beer product. With the rise of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) based genetic engineering it is also possible that a genetically engineered yeast could be utilized to produce low/no alcohol beer, but this attracts significant attention from regulators and consumers in many jurisdictions (Priest and Taylor 2000).

There is seemingly still a large amount of progress possible within the production of no and low alcohol beers, in both engineering processes as well as biological/biotechnological methods. A key concern with conducting research on these at a university scale is the significant space requirement for modern industrial dealcoholisation equipment, with even the smaller units processing 10s of litres of beer a minute. This proves to be a challenge for researchers without large premises and large volumes of product to experiment on, meanwhile the biotechnical approaches encounter issues around regulation of the production of modified organisms and especially their usage to produce food/beverages.

Alternatively, to the progressing of producing products, better methods to assess them before sale represents a potentially large cost saving for brewers, being able to test many smaller test samples without committing significant resources. Due to the historical

significance of beer as well as its large market share of beverage sales analytical work on the molecular composition of beer is common and highly reproduced, from inorganic ion measurements (Minami 2009), organic acid quantification (Affatato et al. 2008), saccharide determination (Batchelor et al. 2015) to volatile organic headspace analysis (Laguna and Sarkar 2017). The chemical quantification of beer and beer products is well defined and while there is still possible advancement it is more often found in instrumental engineering improvements and novel methodologies to detect previously unknown compounds than the simple measurement of a different commercial product.

The use of instruments to measure the lubricating properties of liquids has been common for many years, although originally limited to “hard” tribology for use in design of lubricants and surface modifications in mechanical engineering (Fox et al. 2021; Pitenis et al. 2017) it has subsequently been utilized to measure a range of different products. With current applications of tribology in limb/joint replacement engineering (Cai et al. 2017), analysis of oral medications (Steinbach et al. 2014), measurement of predicted oral properties of wine (Godoi et al. 2017) as well as some initial work in beer measurement (Pang et al. 2020). The development of oral tribology from mechanical tribology has however highlighted some concerns with current tribometers, namely that the forces required for oral tribology are significantly lower, when compared to commonly utilized settings for mechanical tribology (Wang et al. 2024). The commonly utilized 0.5-3 N axial force for oral tribology (Wang et al. 2024) represents around the minimum setting of axial force for several commonly available tribometers, given this proximity to instrumental limits, rates of error can be higher than is desirable. Alternatively to standard tribology, tribo-rheology makes use of a specialized attachment for a rheometer, these instruments are suited to more sensitive axial force

maintenance and have been shown to produce reproducible data in several systems (Wang et al. 2024).

1.2 Research Objectives

The major objective of this project was to validate tribo-rheology as a method for investigating beer and beer related systems. To accomplish this a range of commercial products would be analysed, as well as investigative systems used to elucidate the possible causative molecules for alterations in tribological behaviour and finally using these findings to propose and test possible alterations which may be beneficial to lubrication properties of commercial and experimental systems.

1.3 Thesis Layout

This thesis consists of six chapters, which will cover; the introduction, a review of the currently available literature, three chapters of results, which have been formatted as peer reviewed research papers, details on their publication/submission details can be found on the cover page for each separately. Finally, chapter six contains the overall conclusions from the work as well as proposed future work to continue the work conducted here.

Chapter 1- An introduction to the project as well as its objectives.
Chapter 2- A review of the current literature surrounding low alcohol beer production, analysis and a detailed review of tribology as a technique as well as the theoretical basis behind it.

Chapter 3- An initial investigation into the viability of tribo-rheology to differentiate between commercial beers of different styles, primarily focusing on analysing the alcoholic and non-alcoholic products produced by the same brewery. This was followed by some early testing of some molecules of interest, ethanol, maltose, maltodextrin and sodium chloride, to investigate the possible causes of the observed differences using representative compounds present in standard beers.

Chapter 4- This work utilizes the methodology determined in chapter 3 to perform a detailed analysis of a selection of molecules found in a typical beer and using concentrations taken from literature tests the effect they have individually on tribological behaviour with and without the presence of ethanol. This culminates in the mixture of selected molecules at concentrations found in beer together, to determine if certain chemicals properties are dominant and could have concentration agnostic effects.

Chapter 5- Using molecules which demonstrated possibly interesting properties from chapter 4, this chapter seeks to perform a detailed investigation of the viability of utilizing proteins and amino acids as a post fermentation alteration to beer with a goal of altering mouthfeel properties. This work utilizes molar concentrations and correlation analysis to attempt to explain the observed effects with the goal of assisting in predicting properties of molecules in future.

Chapter 6- A recap of the findings of this project as well as discussion of where future work could lead in this field.

1.4 Publications and Presentations

Some of the results of this project have been submitted or accepted into peer-reviewed journals.

- Holt T, Mills T 2023. Tribo-rheology of alcoholic and non-alcoholic beer. Journal of the Institute of Brewing, 129:164-175. 10.58430/jib.v129i3.31
- Holt T, Mills T (submitted). A microstructure approach to tribo-rheology of beer by molecular composition. The Journal of the Institute of Brewing 2023.
- Holt T, Mills T (submitted). Amino acid and protein manipulations effect on mouthfeel in beer and model systems.

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Chapter 2- Literature Review

2.1 Introduction

The intent of this chapter is to provide a detailed review of the current state of research into the production and analysis of low alcohol beer products, as well an update on progress of tribology/tribo-rheology as a technique.

2.2.1 Beer basic contextualisation

The exact date of the first production of beer is disputed, but it is generally considered that beer has been produced extensively since at least 3500 BCE (Cantrell 2000). The production of beer has however changed greatly since early days. Early beers were produced before the discovery of germ theory and as such held significant superstition around what exactly caused the fermentation process. It is also noteworthy that ancient beers were likely not very similar in character to those produced commonly today, often using local herbs, roots or other flavourings (Hornsey 2003) thus making for significant regional differences in methodology. Indeed, early beers did not contain a currently defining ingredient, hops. These plants were first added to beer in a similar fashion to other locally found plants, but became a common additive in the sixteenth century and are now essentially universal. Despite theoretically very similar ingredients unique beer styles exist around the world as well as within regions of single countries, although due to low demand many historical styles have been replaced by more accessible and or popular ones (Stewart 2006).

More recently, beer has become a very significant commercial product, with multinational brewing conglomerates competing for mass markets across the world while local and craft

breweries frequently produce alternative styles which lack the market volume for larger entities to produce.

Within this complex picture of competing producers, increases in awareness of the health risks of consumption, as well as a change in attitudes towards driving and working under the influence, have contributed to the growth of a new subsector, low and alcohol-free beer. Definitions of these categories vary between jurisdictions, but this work will focus on the United Kingdom's definition under HM Government covered under "Low Alcohol Descriptors Guidance" published 13/12/2018. Under this guidance, low alcohol is defined as "a product with ABV at or below 1.2%" and must be labelled, whereas alcohol free products must contain less than 0.05% ABV and must be labelled with the exact ABV or state it contains no alcohol if none is present. Non-alcoholic, is not a permitted descriptor for alcohol free or low alcohol products, as this is reserved for products which never contained alcohol and must not be used with names that are commonly assumed to include alcohol, e.g., wine, cider, beer (Department of Health and Social Care 2018).

Alcohol (not specifically beer) is a significant cause of preventable mortality in many nations as well as a notable cause of morbidity (Eliasen et al. 2014; Poledнak 2015), this, along with social/societal issues at least in part associated with consumption of alcohol have caused governmental pressure in many jurisdictions on brewers to alter their marketing (Boniface et al. 2023). To comply with regulation, as well as to explore the expanding market for low and no alcohol beer, many new examples have been produced by multinational, as well as local or craft producers. Owing to the unfamiliar nature of these products, their reception by consumers has been mixed (Moss et al. 2022). This is commonly ascribed to the reduced

quality of many low and non-alcoholic beers due to the processing required to remove or to avoid the initial formation of ethanol (Sohrabvandi et al. 2010). This prevailing perception and finding from various analytical profiles of low and alcohol-free beers indicate the need to consider the production methods of these products as this can result in noticeably varied outputs.

2.2.2 Production of Low and alcohol-free beer

2.2.2.1 Dealcoholisation of beer

The standard methodologies for producing alcohol free beers have changed drastically since their earliest iterations. Original products were produced by vacuum evaporating a full strength product to remove the ethanol (Brányik et al. 2012). This process is, however, significantly destructive to other volatile aroma compounds within the product, resulting in very significant losses of esters and higher alcohols, while also yielding an accidental concentration of 2-phenylethanol (Andrés-Iglesias et al. 2016). This results in a drastically different aroma profile for the dealcoholized product, due to the unbalanced removal of aroma molecules, particularly the residual 2-phenylethanol which has a high boiling point of 219 ^{o}C and is known for a strong rose or floral aroma (Larrañaga et al. 2016). During ethanol removal by vacuum distillation, it is possible to collect the lost volatiles which can then be added back to the product, a process known as rectification (Brányik et al. 2012). Here, select portions of the evaporate (those containing less ethanol) can be remixed with the beer to restore some of the lost volatiles, but this is still seen to result in undesirable buildup of 2-pheneylethanol when compared to non-thermal dealcoholisation strategies (Liguori et al. 2015).

Simple vacuum evaporation is therefore rare commercially, due to poor product quality and low throughput (Andrés-Iglesias et al. 2016). Far more prevalent are centrifugal or falling film evaporation. These methods work by producing a thin film layer of beer, which greatly increases the surface area available to evaporate ethanol from and reducing heating times by reducing volumes being heated simultaneously. In both methods, heated steam is flowed against the gravity fed flow of beer, producing a stripping effect as the steam heats and removes the ethanol as it passes (Montanari et al. 2009a). Due to the steams passage over and partially through the beer flow, these methods do risk some oxidation of the beer, but this is generally avoided by deoxygenating the steam flow completely before use. The exact conditions for these processes vary, largely based on the desired final alcohol level, but both require relatively strong vacuums (35-209 mBar absolute pressure) (Brányik et al. 2012) and a significant flow of steam (20-80 m/s velocity). These requirements make the process energy intensive and require high quality vessels to be utilized to prevent implosion, or explosion, during processing. A further concern with thermal methods is a noted increase in the concentration of acetaldehyde within beers subject to thermal stress (Zufall and Wackerbauer 2000). This toxic aldehyde is known to cause cancer (Larrañaga et al. 2016) and is thought to be released as acetaldehyde-bisulphite complexes are oxidized by heat and existing oxygen (Zufall and Wackerbauer 2000). While pure acetaldehyde is carcinogenic and highly reactive, its health effects in beer have not been extensively studied, as for alcoholic beers the effect of ethanol is difficult to separate from any other negative health effects. However, it has been associated with an increased risk especially in areas where acetaldehyde levels are high (Lachenmeier et al. 2009).

In response to the concerns raised with even limited heating of beer, non-thermal methods have been developed to remove ethanol. These are based around the use of partially permeable membranes, but with various mechanisms of action. Reverse osmosis is a commonly used method for purification of water for laboratory use, but it can also be utilized for a wide range of selective extractions. Within beer dealcoholisation, the beer and stripping solution are held at 1-5 ^{o}C to reduce thermal stress (Catarino et al. 2007) and passed under pressure against a stripping solution separated by a membrane. Membranes for this purpose can be produced from a variety of materials, cellulose acetate, polyamide (Catarino et al. 2007) and polypropylene can all be utilized (Russo et al. 2013). While the choice of material will alter the exact extraction efficiency, durability and life span, the primary factor in membrane selection is often pore size (Pilipovik and Riverol 2005) or cost, where the rate of membrane degradation is a key factor.

A primary advantage of reverse osmosis when compared to thermal techniques, is the preservation of thermally labile molecules, particularly antioxidants and polyphenols (Russo et al. 2013), while a significant disadvantage is the difficulty in achieving alcohol levels lower than 0.5% (Catarino et al. 2007; Brányik et al. 2012). This difficulty is a by-product of the mechanism of action being linked to a concentration gradient between the product and the stripping fluid, with rates of transfer slowing as the concentration difference is reduced. It is also noted that reverse osmosis requires a significant water supply, generally twice the volume of product is needed in stripping solution (Russo et al. 2013) adding another expense to the process, although this water can be recycled after reprocessing.

A concern unique to reverse osmosis dealcoholisation is the potential loss of colour (Alcantara et al. 2016) as the molecules providing the pigment to beers can also be lost over the membranes if pore sizes are sufficiently large.

In contrast to reverse osmosis dialysis presents a far more passive mechanism for ethanol removal. Similarly to reverse osmosis, stripping fluid is passed against the product, which sits within a partially permeable membrane allowing diffusion of ethanol from the product (Moonen and Niefind 1982). As transmembrane pressure differential is not a significant factor in dialysis efficiency, minimal pressures can be used, reducing costs and safety requirements (Moonen and Niefind 1982). However, dialysis is less commonly utilized than other methods, primarily due to its lower efficiency, often not being able to reduce ethanol concentrations sufficiently to meet statutory requirements (Liguori et al. 2015).

Pervaporation represents a possible remedy for ethanol removal based defects. This process utilizes a hydrophobic membrane separating the product from a strong vacuum (Takács et al. 2007), volatiles are then drawn through the membrane and condensed on the other side. This extract can be used to re-flavour subsequently dealcoholized beer, or sold separately as an industrial concentrate. While pervaporation has been used to produce low alcohol beers (Catarino et al. 2009) and wines (Takács et al. 2007) previously, this is not common industrially. Pervaporation requires the lowest pressures possible, generally 1 mBar provides optimal extraction, presenting significant costs for vacuum production and vessel quality (Catarino et al. 2009).

2.2.2.2 Low alcohol brewing

In the face of high costs in equipment and energy, a natural alternative to removing ethanol from beer is to produce products with low or no alcohol to begin with. This approach can take several forms, broadly; reduced alcohol using standard yeast varieties or reduced alcohol using alternative yeast strains. Naturally limited fermentation has traditionally been used to produce small beers, a historically important source of calories and storable water throughout history (Roberts et al. 2012), but these low gravity beers have largely fallen out of favour more recently. Artificially limited fermentation, can however, be used to produce lower alcohol beers by reducing the efficiency of fermentation, for example a wort can be cooled to close to 0 ^{o}C or the yeast cells physically removed to arrest fermentation (Brányik et al. 2012). This process is not commonly utilized for commercial production of low alcohol beer, due to poor final characteristics (Willaert and Nedovic 2006) and potentially high cost if removal of yeast cells is required.

Alternatively to the more basic arrested fermentation, cold contact can be utilized, this process uses a similar principle, but more specific conditions. For example, wort is brought to ~pH 4 using exogenous lactic acid with yeast pitched at -0.5 ^{o}C (Schur and Sauer 1990). This process has been reported to yield a beer with as low as 0.05% alcohol (Schur and Sauer 1990), but has also been noted to yield high levels of the aldehydes 2-methylbutanal and 3-methylbutanal (Perpète and Collin 1999b) which are known to contribute to malt odour (Perpète and Collin 1999a). These aldehydes would normally be removed by enzymatic action from yeasts, but, due to low temperatures required for the process their removal is significantly limited, yielding higher than desirable levels. Aldehydes are also known to bind

to polyphenols, potentially protecting them from enzymatic activity even if conditions were more optimal. This has, however, been suggested as a mechanism for their removal using polyvinylpolypyrrolidone (PVPP) filtration, which could remove the polyphenols along with the bound aldehydes (Perpète and Collin 2000).

Beyond manipulation of the fermentation conditions, manipulation of the yeast themselves is also considered a key area for research into production of higher quality low and non-alcoholic beers. Early attempts at genetic selection or manipulation focused on alcohol dehydrogenase as an obvious target. This gene is responsible for the production of ethanol from ethanal (Raj et al. 2014) so presents an initially enticing strategy for reduction or removal of ethanol production. Unfortunately, ethanal is highly undesirable, especially at unnaturally high levels and yeast with this deficiency are often created by genetic engineering-based methods CRISP etc. meaning they are not considered suitable for food production in many jurisdictions.

Artificial genetic modification of yeast has been further refined, with modifications further up the synthesis pathway, thus avoiding the build-up of ethanal and instead shuttling carbon into a less harmful product, glycerol (Nevoigt et al. 2002). While this shuttling mechanism appears appealing, it shows a low efficiency for reducing alcohol content, only typically yielding 18% reductions, while significantly increasing diacetyl, acetaldehyde and acetoin (Nevoigt et al. 2002). Further modification was then attempted, by modifying genes related to acetaldehyde production (Ald6) (Remize et al. 2000). This still left acetoin levels higher than desired, so even further modification was used to overexpress a plasmid containing 2,3-butandiol dehydrogenase (Ehsani et al. 2009). With so many modifications, regulatory

approval becomes more challenging, as describing the mechanism and function of each modification adds challenges for any commercial or scale use. To avoid such harsh regulation, it is possible, but far less precise, to allow a population of yeasts to spontaneously mutate. Due to the very large populations being considered even with low mutation rates it is mathematically likely that many useful or potentially useful mutants are created. It is, however, noted that this sort of mechanism is highly imprecise and often the phenotype is measured rather than being able to directly identify the genetic level alteration (Strejc et al. 2013). This imprecision leaves a much greater risk of off-target mutations, or unknown silent mutations which could cause issues under specific circumstances, or that the spontaneous mutation may simply revert naturally, or when exposed to specific conditions.

Alternatively to inducing or searching for mutant *Saccharomyces cerevisiae* strains, entirely different strains can be utilized. This is already common in standard brewing with *S. pastorianus* (formally *carlsbergensi*) being utilized for the production of larger beers (Walther et al. 2014). In terms of low alcohol brewing, several strains have been investigated and found to have potentially desirable characteristics. Primarily this centres around inability to ferment maltose, while still yielding beer like characteristics in terms of volatile aromatic and other non-ethanol molecules. *Saccharomyces ludwigii* has demonstrated potential as a candidate for lower than standard but not <0.5% alcohol brewing, although this required combination with high temperature mashing (De Francesco et al. 2015).

More radical strategies have also been attempted experimentally, with several non-saccharomyces species showing promising results. *Torulaspora delbrueckii* shows particularly favourable biological characteristics, being both salt and ethanol tolerate, unlike many non-

saccharomyces species (Araújo et al. 2005). This makes it more suitable physiologically for utilization in potentially osmotically stressful environments such as wort. Conversely, most strains produce alcohol by volume around 2.6% (Canonico et al. 2016), which is lower than would be expected for standard yeasts, but still significantly above any desirable legal descriptor. Other studies have found strains of *T. delbrueckii* produce less than 1% ABV products (Michel et al. 2016). It was also noted that flocculation characteristics are overall poor for this species (Canonico et al. 2016; Michel et al. 2016), but some strains have been shown to flocculate. Flocculation being a desirable characteristic for traditional beer production, due to reducing the requirement for filter/fining of products for sale, although with growing acceptance for "fog" style yeast within the craft market, this may prove less of a concern moving forwards.

Zygosaccharomyces rouxii has also been investigated for potential brewing applications, strains are seen to have an ability to metabolise ethanol, which would be highly desirable as a method to remove existing alcohol without the need for chemical processing (Sohrabvandi et al. 2010). This ability is however, only present under aerobic conditions, which would likely result in oxidative stress to the product, reducing shelf life. Despite this limitation, some brewing trials have been conducted, but resulted in higher than desirable levels of diacetyl, acetaldehyde and pentanedione (De Francesco et al. 2015), even when used as a secondary fermenter, which would have been the obvious application for use as an alcohol removing yeast. However, this ability could represent a potential target for future genetic investigation, either by attempting to identify a mutant which does not produce such high levels of vinkyl diketones and aldehydes, or by direct genetic modification of existing strains.

Unlike *Z. rouxii*, *Wickerhamomyces anomalus* has been utilized for small scale commercial applications, primarily in wine production (Sabel et al. 2014), although it has also been utilized for increased volatile flavour compounds in Baijiu (a distilled clear alcohol frequently made from sorghum or rice) (Zha et al. 2018). Examined strains were found to produce high levels of ethyl acetate and butyrate, but still generated 1.5% ABV, which, while lower than the 4% from *S. cerevisiae* fermentation of the same material (Sabel et al. 2014), is still not within any specific legal category. This, similarly to *Z. rouxii*, could present an avenue for modification of further investigation into alternative strains or conditions which may be able to produce the desirable esters, while producing lower levels of ethanol. *W. anomalus,* unusually, is classified as a toxic killer yeast, this while problematic for marketing, is not a health or regulatory concern, as the toxin is only harmful to other microorganisms (Farkas et al. 2012). The presence of this toxin could in fact, present a boon for brewing usage, as it is highly toxic to the common spoilage yeasts, *Dekkera spp.* and *Brettanomcyes spp.* (Comitini et al. 2020) and could be used to supress traditional yeasts if *W. anomalus* were to be used as a secondary fermentation.

It has also been found that a strain of *Pichia kluyveri* was able to produce cider (Saerens and Swiegers 2016a) and beer with an ABV of 0.1%, while producing high levels of aroma molecules (Saerens and Swiegers 2016b). Counterintuitively, the species is also utilized in dual tequila fermentation, specifically for its ability to produce alcohol more rapidly than standard mono-fermented products, while still producing high levels of esters and higher alcohols (Amaya-Delgado et al. 2013). It is also used in wine production, where, like *Wickerhamomyces* it exhibits toxic killer characteristics and is known for only fermenting glucose (Contreras et al. 2014). This desirable sugar fermentation profile is potentially countered by the production of unusual (in beer) thiol molecules. The thiols 3-

mercapotohexyl acetate and 3-mercaptohexan-1-ol are commonly seen in Sauvignon Blanc produced in New Zealand and is linked to *P. kluyveri (*Anfang et al. 2009) but their customer perception in beer is yet to be determined.

2.2.3 Analysing beer

2.2.3.1 Ethanol determination

The analysis of the content of beer and other alcoholic beverages has historically been relatively advanced when compared to other food stuffs. This is generally due to taxations applied to alcohol content, meaning requirements for the reporting of alcohol content have existed in England and Wales since 1643 (Yeomans 2018). Early excise duty often did not distinguish alcohol by volume, simply that alcohol was present or not. More detailed taxation was introduced subsequently and has grown into a complicated system, based upon the output of the brewery and the alcohol by volume of its products, with those over 7.5% incurring a greater rate of taxation than those between 2.8% and 7.4%, while beers below 1.2% do not incur any alcohol tax (Revenue&Customs 2023). As such determination of accurate alcohol by volume measurements is key within commercial brewing and has been considered important for some time. This requirement for alcohol measurement has brought about several generations of methods, from very early to very modern, although many of the oldest techniques are still utilized in some situations today, due to cost concerns and simplicity of use.

The oldest methods for determination of ethanol in beer are generally related to the physical properties of ethanol compared to water. These methods utilise its boiling point for distillation, density for hydrometers and refractive index for refractometers. Of these,

distillation has fallen out of favour, primarily due to regulation around distillation of spirits often being more stringent than brewing and the low accuracy of the method. Hydrometer and refractive index determination are still commonly used in modern breweries and are both considered acceptable for determination of ethanol for taxation purposes (HMRC 2016). Hydrometers have however been noted for their inaccuracy and, due to temperature specificity, can prove challenging to yield accurate results (Speer 1802), this has been a concern discussed even early into modern brewing (Spedding 2016). The hydrometer method works based upon the change in density of the wort during fermentation. This introduces a significant downside, in that if the starting density of the wort is incorrectly or inaccurately measured it becomes impossible to calculate the alcohol content (Speer 1802). Refractometers, on the other hand, are capable of back calculating original gravity and alcohol content, given only the refractometer reading and the current gravity (Brown 1977) the latter being required for removal of possibly interfering effects from dissolved solids. Although this technique is somewhat more complex than simple gravity comparison, it does allow for third party quality control of products where the original gravity is in doubt. It is, however, noted that the accuracy is generally ± 0.3%, a level which could be acceptable for smaller breweries, but is largely undesirable when bigger scales are considered (Spedding 2016).

More modern methods for determination of ethanol are generally either chemical or chromatographic in nature. Biochemically it is possible to determine ethanol by use of alcohol dehydrogenase and diphosphopyridine nucleotide (DPN) (Bonnichsen 1965). This method is commonly utilized and is available as a ready-made kit, which is noted for its specificity, ignoring many common interfering molecules (Upperton 1985). Alternatively, the

product can be distilled dry and the distillate reacted with hexavalent chromium in the form of potassium dichromate and the reduction of 4+ to 3+ ions is measured by visible wavelength spectrophotometry (Caputi et al. 1968). This redox based method is not popular, due to the high toxicity levels of chromium ions and the need for distillation.

Chromatography represents a high throughput and reproducible method for the analysis of ethanol and a range of other molecules of interest, often simultaneously. High performance liquid chromatography (HPLC) has been utilized to measure ethanol, sugar and organic acids in a simultaneous quantification (Doyon et al. 1991; Lefebvre et al. 2002) but, due to high initial costs and required expertise, HPLC (similarly to all chromatographic methods) is generally limited to external contractors and large breweries. Gas chromatography, with or without mass spectrometry, has also been shown to reliably quantify ethanol with minimal sample preparation (Upperton 1985) and is also widely used in volatile aromatic analysis, with quantification in the parts per million range achievable (Pontes et al. 2009). The technique is readily applicable to low alcohol products, where high variation in accuracy is of greatest concern.

2.2.3.2 Saccharide analysis

Saccharides represent a key class of molecule for fermentation, as well as final product labelling and consumer perception, as such both simple and complex saccharides are extensively measured within brewing. Traditional methods often involve reaction with sulphuric acid and anthrone (9(10H)-Anthracenone) which produces a green colouration detectable by visible light spectrophotometer (Yadav et al. 1969). This method can be combined with paper chromatography to provide quantification of separated saccharides, or

used alone to provide total values (Yadav et al. 1969), the relative simplicity of this method means it is still utilized in some instances.

Given the requirement for separation of the multiple sugars found within beer/wort, chromatography is more commonly utilized, most commonly liquid chromatography, either High Performance or Ultra High-Performance Liquid Chromatography (UHPLC). Due to the water soluble and chemical nature of saccharides, standard carbon ligand modified silica columns offer very poor retention, thus separations require specialist column materials, increasing costs. The bonds present within saccharides also do not lend themselves easily to detection by normal HPLC methods. With poor UV absorbance at most wavelengths, forcing the utilization of ~210 nm, this increases background and interference from the significant number of compounds found within beer.

Refractive index detectors are better suited to carbohydrate analysis and are standard practice for commercial quantification of saccharides (Castellari et al. 2001), it is also possible to quantify organic acids and ethanol during this methodology, as mentioned in 2.2.3.1 (Castellari et al. 2001).

UHPLC offers a shorter analysis time, which is a significant advantage when many samples must be analysed (Fountain et al. 2009), but comes at significantly increased initial investment in both columns and instrumentation. Mass spectrometry can also be included for additional sensitivity, but, given the high abundance of saccharides in beer is not generally required (Araújo et al. 2005). It is also possible to analyse smaller saccharides by GC/GCMS, but, due to their non-volatile nature, derivatization must be included, which given

the added complexity and cost of reagents is not common, but has been successfully conducted previously (Otter and Taylor 1967).

Vitamins provide an important component of beer, providing both improved shelf life via anti-oxidant capacity reducing risk of oxidative stress and health benefits for consumers with vitamin C and B vitamins (Donovan and Hanke 1936) reducing a range of morbidities. This vitamin content has largely been overshadowed by the negative effects of alcohol, but, with the rise of no and low alcohol beer, it has become more relevant. Historically, vitamins, particularly B6 (pyridoxine) were measured by bioassay (Hopkins and Pennington 1947) although this method is noted for its variability and bioassays in general have become less common as chromatography and other methods have advanced. Meanwhile, vitamin C was classically quantified by titration against 2,6-dichlorophenol indophenol (DCPIP) (Musulin and King 1936). Modern UHPLC methods are significantly faster and have greater reproducibility and accuracy (Spínola et al. 2012), again with the disadvantage of increased initial cost. Other vitamins were not historically measured regularly, but now can be quantified simultaneously by HPLC or UHPLC, with or without mass spectrometry (Mendiola et al. 2008).

2.2.3.3 Volatile Organic Compound analysis

Volatile Organic molecules are known to be primarily responsible for the aroma of food and beverages, thus their analysis has been extensively studied, although largely more recently due to challenges in their measurement without modern chromatographic techniques. Given that VOCs are all volatile, gas chromatography represents by far the most common method

for their analysis, although exact methodology varies, most commonly headspace extraction is utilized, either by direct injection of gaseous headspace (Murakami et al. 1987) or by solid phase microextraction (SPME) (Jeleń et al. 1998). Both methods offer advantages, with simple headspace analysis being faster per replicate, due to no fibre incubation time as well as more suitable for manual sampling, but high levels of inter-laboratory variability have been noted (Dupire 1998). SPME has been shown to be more reproducible (Shirey 2012), but more time consuming per replicate, due to requirements for fibres to adsorb molecules and equilibrate for effective analysis. The requirement for holders and fibres also represents an initial investment cost.

In addition to static SPME, several more complex head space absorbative methods have been developed, most simply HeadSpace Sorptive Extraction (HSSE) and Stir Bar Sorptive Extraction (SBSE) which provide an active element to extraction, where the adsorptive material is magnetically stirred to improve extraction speed (Bicchi et al. 2002). HSSE features gas phase stirring, while in SBSE the adsorptive material is immersed in the liquid. These techniques are less common than simple static headspace fibre extractions, but can offer faster equilibration times, although costs are increased due to more specialist devices being required and immersing the fibres can risk damaging them with non-specific binding (Richter et al. 2017). Desorption of analytes from these techniques is also more complex, as the magnetic elements cannot simply be inserted into the injection port of an unmodified GC as SPME fibres or headspace needles can be, they require desorption into a cryotrap which can then control the entry of molecules into the chromatography system (Richter et al. 2017), again increasing complexity and cost.

While most VOCs are capable of analysis by GC in their native state, some, particularly acetaldehyde are so highly volatile that many chromatography systems struggle to retain then sufficiently for quantification (Wu and Hee 1995). Although this issue has been reduced with the further development of highly polar stationary phases for GC, it is possible to use (O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA·HCl) to improve retention by providing on-fibre derivatization of aldehydes to increase their retention on lower polarity phases (Wu and Hee 1995). Otherwise, derivatization for VOC analysis is not common, with most compounds able to be separated relatively easily by standard temperature gradients.

2.2.3.4 Protein and amino acid analysis

Other key components of beer are proteins and amino acids, these have previously been quantified by a range of assays, with Bradford, Coomassie brilliant blue dye binding (CBB), Bicinchoninic acid (BCA) (Siebert and Lynn 2005) and Kjeldahl assays (Devani et al. 2020) all utilized previously. More modern methods for available nitrogen, as well as protein, have also been developed using ninhydrin in high throughput methods (Abernathy et al. 2009). These assays only provide a quantification for protein content and do not suggest anything towards the composition of that protein, this requires some separation technology to be applied. Traditionally, polyacrylamide gel electrophoresis (PAGE) has been used to give an indication as to the breakdown of the molecular weight of the proteins within a beer sample (Huston et al. 1986). This method improves upon the simple quantification provided by the previously mentioned assays, but, being purely based on size cannot give insight into the functions or origins of proteins. The addition of western blotting can allow for identification

of specific proteins or sources of specific visible bands (Picariello et al. 2015), but increases the complexity of the method significantly.

A common strategy for proteomic analysis in many systems is HPLC-MS/MS, which is able to elucidate the structure of the proteins which are separated during analysis. This capacity allows for identification of unknown proteins, for which no antibody may be available, thus could not be identified by western blot (Wessels et al. 2009). This level of granular detail in protein composition is not generally required for standard commercial brewing, but could be of interest in novel systems or those which have been modified in some way, to ensure those alterations have not changed the protein profile. They are also important for confirmation of any intentional (or unintentional) genetic changes in yeast altering protein expression that are used for brewing, through CRISP or other methods. The high cost of LC-MS/MS systems limits their utilization for many applications, whereas gel electrophoresis-based methods are readily available to even medium sized commercial operations.

2.2.3.5 Inorganic ion analysis

The inorganic composition of beer is tightly controlled commercially, commonly by reverse osmosis or ion exchange both of which are capable of controlling starting water conditions (Solt 1984). This is additional step is based upon safety, in that containing hazardous metal ions is a health concern for consumers (Reilly 1973; Soares and Moraes 2003) as well as quality/perception, as alterations to ion composition can alter perception of flavours (Hough et al. 1982; Montanari et al. 2009b). Measurement of inorganic ions is therefore considered important at all levels of beer making, from home brewing to multinational operations.

Primarily this is controlled at the starting water level for most operations, with water providers conducting analysis of the tap water before providing it. This original value can then be altered as desired for a specific product. Larger operations may test the tap water in house or externally to ensure it is as described (Montanari et al. 2009b). The methodology for measuring inorganic solutes in water is relatively well established, with a range of methods utilized. Atomic absorption spectroscopy (AAS) has been utilized for over 30 years to measure ion content in beer and water (Ybáñez et al. 1989) and is still commonly used (Silveira et al. 2023). Atomic absorption spectroscopy identifies elements by exciting them with radiation and measuring the spectrum of light given off (García and Báez 2012). It is able to quantify and identify up to 62 metal ions from a single sample, but, due to initial costs and expertise requirements are not available in house to smaller operations. This range of metals includes effectively all relevant ions for brewing purposes, as well as possible contamination ions.

Alternatively to AAS, inductively coupled plasma-mass spectrometry (ICP-MS) can be utilized for the determination of many metal ions in a range of samples with detection possible down to pg/L (Chemnitzer 2019), although this limit is specific to each element due to ionization energies. The extreme sensitivity of modern ICP-MS instruments is largely unnecessary for routine analysis of common components of beer, but can be useful in quantifying low abundance contamination elements, such as heavy and rare earth metals (Mahmood et al. 2012).

For quantification of non-metal ions, ion chromatography is most commonly utilized, as it is able to determine levels of chloride, sulphate, phosphate (Buckee 1995), bromide, nitrate

and fluoride (Boyles 1992) within beers. The combination of either AAS or ICP-MS with ion chromatography is capable of determining the inorganic composition of beer with high levels of accuracy and reproducibility. It has also been found to be possible to simultaneously measure organic acids and inorganic ions using gradient ion chromatography (Boyles 1992).

2.2.3.6 Physical Analysis

A less commonly studied area of beer analysis is the physical properties of the products. While density is frequently measured, generally for the determination of ethanol content (see 2.2.3.1), other physical properties are rarely measured at a brewery level. Some work has been conducted on viscosity, largely in relation to grain derived β-glucans (Sadosky et al. 2002; Lee 2008) and recently tends to focus on abstract measurement. Although previously viscosity was considered experimentally around mouthfeel (Ragot et al. 1989), instrumental measurement of beer mouthfeel is uncommon. Previous work has utilized tasting panels to assess the mouthfeel properties of products and experimental solutions (Langstaff et al. 1991). While tribological methods are utilized in other alcoholic (Laguna et al. 2017; Wang et al. 2020) and non-alcoholic systems (Batchelor et al. 2015; Morell et al. 2017), but very little tribology has been conducted on beer or beer related systems (Fox et al. 2021; Holt and Mills 2023). As such this area represents an interesting field for further study, especially in relation to experimental low alcohol beers which may not be considered safe for human panels to taste due to unconventional production methods, or non-food safe laboratory scale manufacturing.

2.2.3.7 Tribology and Tribo-rheology

Tribology and tribo-rheology have been used to make instrumental assessments of oral perception in a range of systems, these include full products; dairy semi-solids (Godoi et al.

2017), oral medications (Batchelor et al. 2015), wine (Laguna et al. 2017) and beer (Fox et al. 2021) (Holt and Mills 2023) as well as experimental systems of fluid gel (Mills et al. 2013) and pure inorganic salt systems (Garrec and Norton 2012). The field is initially separated into hard and soft tribology, soft tribology tends to deal with investigating human perception of liquids, solids or semi-solids, while hard tribology deals with mechanical concerns in industrial applications of lubricants, for example in combustion or jet engines, although the definition is not entirely officially defined with some crossover between the two existing in bio-medical applications among other areas (Affatato et al. 2008). Indeed even the perception of what is considered a hard surface will vary depending on the field (Pitenis et al. 2017). Due to its increased relevance to food/beverage systems this review will focus only on soft tribology aspects and the variation within those systems.

Within soft tribology there is an immediate split in methodologies, between the use of tribometers such as Mini-Traction Machines (PCS instruments) and THT (Anton-Paar) or tribo-rheology, which utilize specialist attachments for standard rheometers available from a range of manufacturers e.g. TA Instruments, Anton Paar, Netzsch. The schematic differences are presented in Figure 1, showing a PCS Instruments MTM2 compared to a TA Instruments Discovery Hybrid Rheometer 1.

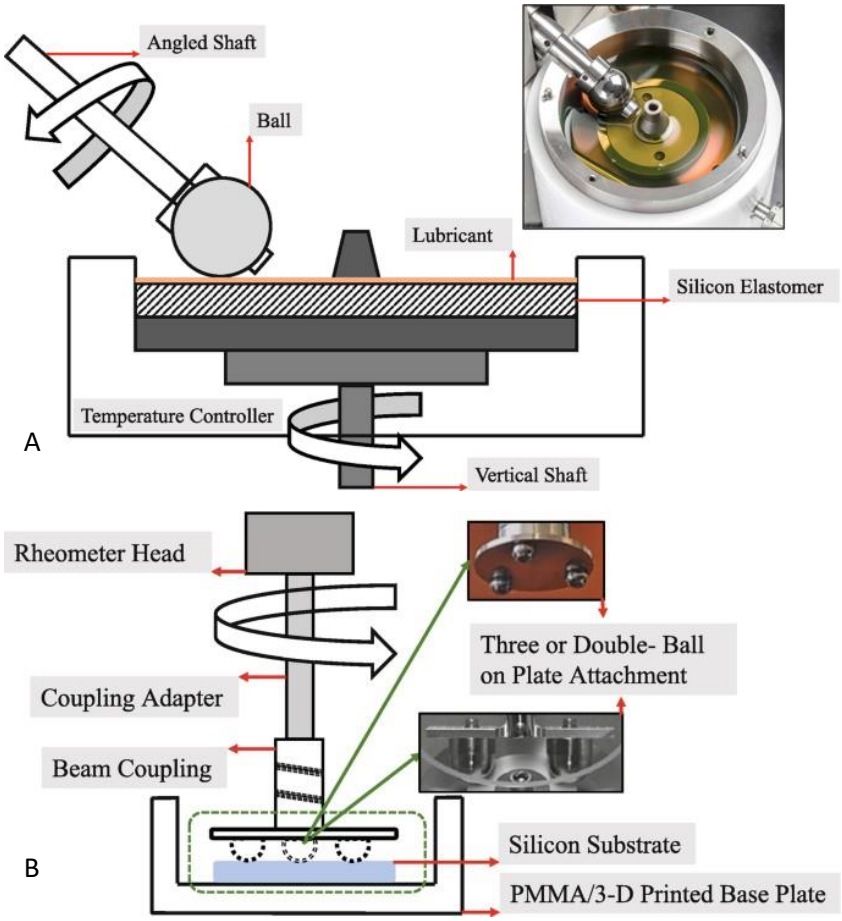

Figure 1. Schematic diagram of (A) MTM2 tribometer and (B) DHR1 tribo-rheometer adapted from (Wang et al. 2024)

The use of tribo-rheology is attractive, as it makes use of instruments many food engineering departments already have access to for use in gel or semi solid research. In contrast, tribometers are able to more readily suitable for hard tribology work and are capable of producing much greater downforce (~75 N) over a wider range of temperatures as standard (ambient-150 ^{o}C). Within food and beverage applications, these advantages are limited in use, as very high temperatures are not relevant to oral perception and measurements of oral force in feeding range from 2.10 to 32.43 N (Hiiemae and Palmer 2003). There is also a mechanical difference between standard tribometers and tribo-rheometers, whereby the contact in tribometers is generated by rolling a surface against another, while tribo-rheometers utilise a sliding motion. This means the entire contact surface remains in contact

at all times when sliding, while the rolling of the ball varies which part of the surface is actually in contact during the rotation. This difference does not present a specific advantage or disadvantage to either technique, but can cause higher friction based on a higher constant area of contact. As much of the food related tribometry is conducted at relatively low normal forces (<10 N) the improved low force range of rheometers can be advantageous, allowing for higher force sensitivity (~0.005 N depending on model).

Once an instrument has been selected; further methodological decisions remain. In soft tribology, several surfaces can be selected from, these include but are not limited to surgical tape (Nguyen et al. 2016), silicone elastomer (Mills et al. 2013), polydimethylsiloxane (PDMS) (Sarkar and Krop 2019), pig tongues (Ranc et al. 2006) and more complex simulated human tongues (Wang et al. 2021). These soft surfaces intend to mimic the uneven topography and approximate rigidity of human soft oral surfaces, while the harder surface (often glass or stainless steel) replicates the hard palate present in vivo. Each surface offers advantages and disadvantages; with artificial options frequently being more reproducible, but less representative of real world conditions, while non-human tongues offer a biologically more similar, but homogeneous pool of surfaces. A significant concern with animal tongues is their physiochemical properties are changed following the death of the animal. This often requires significant processing to return them to a more lifelike state (Ranc et al. 2006). Variation between individual animals is also significant and, while this is relevant to human perception of oral properties, it is not desirable within a tribology environment. When combined with ethical/health concerns, these issues make biological tongue tribology relatively unusual.

Once a surface is selected, another significant experimental choice is presented. Saliva is known to be a key modifier of oral experiences in humans (Morell et al. 2017; Laguna et al. 2017) and as such the inclusion of and type of saliva to use is important. Many studies do not attempt to simulate saliva. This is frequently due to the added complexity of their inclusion as well as challenges around how to add saliva consistently. If saliva is chosen to be included, the options are to utilize real human saliva, often taken from volunteer donors or to produce an artificial saliva analogue. Real saliva is easily available, but presents consistency issues, with composition varying between individuals and between days or times of day (Dawes 1972). The use of volunteer saliva also presents health concerns, where diseases can be transmitted by the handling of unscreened saliva (Slots and Slots 2011), while screening of saliva represents an additional cost to the research. Alternatively, artificial or analogue saliva can be utilized. This represents a more consistent option, as it can be produced periodically to a known standard. Model saliva does however bring back issues related to individual variation. Studies of authentic saliva have found varying compositions (Dawes 1972; Nasidze et al. 2009) and linked this variation to perception of food products (Neyraud et al. 2012). Given this variation, picking a specific make up to be the analogue is challenging.

Once all physical conditions are selected, experimental parameters remain to be chosen; for example the previously discussed normal force. Measurements of forces in oral processing vary greatly between studies and individuals (Rudge et al. 2019) as well as between different food/drink products (Sethupathy et al. 2021). This makes selection of a single value for oral forces both inaccurate and arbitrary, but also necessary for the methodology. The generally stated range for tongue force exertion is 0.01-90 N (Miller and Watkin 1996) with average surface movements of 2.10-32.43 mm/s, but individual results were as high as 305.67 mm/s

in early phases of transport (Hiiemae and Palmer 2003). This makes the selection of a single normal force challenging, as, while a sweep of speeds can be completed, or a sweep of forces at a constant speed, doing both exponentially increases the time per sample, reducing the quantity of data that can be generated. As such, a single value for downforce is generally selected and a rate sweep of slider/rolling speed is used, this generates a friction over speed graph, from which areas of interest can be selected. This coefficient of friction over speed graph is referred to as a Stribeck Curve and are commonly used to compare lubricity of systems.

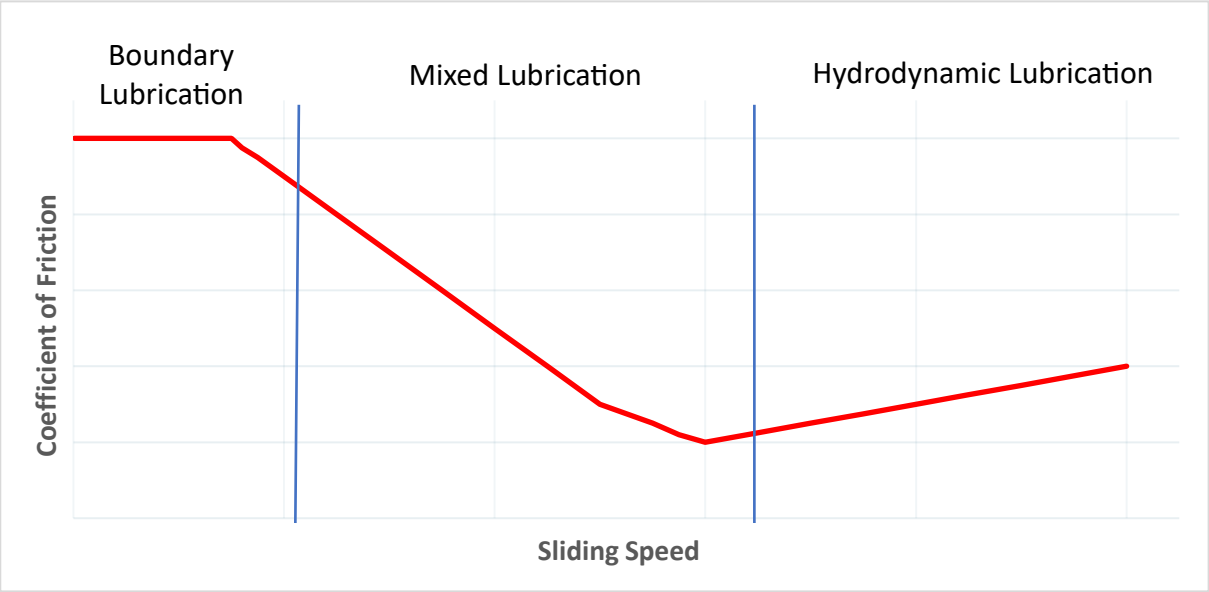

Figure 2. Schematic diagram of a Stribeck Curve, demonstrating the behaviour of different lubrication schemes.

The test speeds, either rolling or sliding are broadly categorised into three portions, these being based on the dominant deciders of friction at those speeds (see Figure 2), those being hydrodynamic, mixed and boundary (Rudge et al. 2019). At very high speeds, the bulk properties of the fluid are considered to be dominant. Here, a thin film of fluid provides lubrication between the surfaces, the thickness and efficiency of this lubrication is dependent on the system being tested (Sethupathy et al. 2021). As speed reduces, a mixed

lubrication system is established, whereby the fluid layer still provides significant lubricity, but some asperity contact is established between surfaces. Further reductions in speed reduce the contribution of the fluid layer, causing an increase in friction as greater surface contact is achieved (Cassin et al. 2001). With sufficiently low velocity, the boundary region is reached. Here lubricant film is not present and only asperity contact drives friction, this results in high friction forces with significant wear of surfaces. The specific velocity where a given behaviour is found varies depending on several factors, in hydrodynamic regions viscosity is a key modifier, determining the ability of the fluid to entrain and produce films for lubrication (Sethupathy et al. 2021). This film formation is countered by the roughness of the surfaces being tested, as, to prevent contact, the films must be sufficiently deep to cover both surfaces' imperfections, thus preventing asperity contact. As such the velocity to achieve this effect varies with the ability of the fluid to form films as well as the surfaces level of roughening. The nature of animal tongues and palates presents a significantly less microscopically consistent surface than commonly used models (PDMS and silicone), with roughness values between 50 and 160 µm, but with significant variation over the tongue surface as well as between individuals (Wang et al. 2019). In contrast to this, poured and gravity flattened PDMS surfaces would be expected to exhibit very little, to no surface texture, while faces set against a mould would carry some of the texture of that surface into their final set shape. This roughening would be highly dependent on the chosen mould material, with milled metals or 3D printed resin or poly lactic acid moulds likely to show significant differences in surface texture. The outcome of this difference in roughness is the speed range where hydrodynamic effects will dominate, with rougher surfaces more prone to asperity contact as a thicker film of fluid is required to prevent contact. As such it could be predicted that a flatter artificial surface would experience hydrodynamic forces over a wider

range of test velocities than a more highly textured surface would. Due to both physical and chemical differences between surfaces, direct numerical comparison between studies can be challenging, as, even the same material formed in a different mould is likely to have a significantly different surface topography, resulting in different hydrodynamic prevalence and asperity contact.

Due to bulk fluid properties determining hydrodynamic effects, changes to the fluid which are not significant enough to noticeably alter viscosity or other physical property do not, in general produce changes to friction as fluid motion is being governed by Continuity and Navier-Stokes mechanics (Gropper et al. 2016). This, coupled with the high velocity required to achieve purely hydrodynamic effects, often results in lower relevance to simulated oral systems, as the forces required to show purely hydrodynamic lubrication are rarely present (Hiiemae and Palmer 2003). Once velocity is sufficiently low to allow for asperity contact, the role of the lubricant changes, from purely keeping the surfaces separate to reducing the friction and wear experienced by the movement of one against the other. In hard tribology the concept of tribofilms or “anti-wear” films is common (Thrush et al. 2021; Rai et al. 2016), these thin films of dissolved additive bond to surfaces, producing a consistent layer of added material, separate from the original surface. It is noted that extreme pressure films are also possible, but are only formed under forces not relevant to soft tribology (Willermet et al. 1995). Their anti-wear capacity is as a disposable layer of material, which can be worn away and potentially deposited again, depending on conditions, or at least provide a sacrificial layer reducing wear on the original surface (Willermet et al. 1995). The non-permanent nature of these films means their loss can be measured over time as an increase in friction without any changes in conditions, as the underlying surface which is more strongly bonded

is less likely to yield, resulting in a higher friction as the two surfaces wear against each other, rather than the weaker tribofilm.

These tribofilms can be stabilized by several interaction types, with varying strengths of bonding. Adsorption represents the least permanent attachment mechanism being a weak intermolecular force interaction by Van Der Waals or electrostatic attraction between oppositely charged or polarised groups (Ismail and Bagheri 2017). These films are of low strength and are easily abraded by shear, while being separated into specific and non-specific interactions, primarily adsorption effects are non-specific being mainly related to classic Van Der Waals. Fully chemically bonded moieties are also possible (Willermet et al. 1995) representing permanent chemical bonds between the film forming molecule and the surface. These offer a significant improvement in stability due to the now intramolecular forces (Simic and Kalin 2013). Primarily organic molecules would be expected to bond and interact with the carbon present in stainless steel alloys, or to hydroxides of these carbons (Simic and Kalin 2013), while inorganic compounds are seen to interact with the inorganic elements present within the surfaces and have been seen to provide effective tribofilm formation (Dubey et al. 2022).

The smoothing effect of deposition of material onto tribofilms has been shown to reduce friction as well as wear (Zhmud et al. 2014). Similar films have been visualised in emulsion tribology on PDMS disks (Lim et al. 2022) and it seems likely that this effect is increased in prevalence with an elastic polymer surface present, allowing a wider range of substances to bind to one or both surfaces due to the increased heterogeneity in the system.

Primarily food/beverage tribology is conducted on complete commercial products or model systems. This is likely due to the complexity of the systems utilized and the way in which products are perceived, but makes it challenging to assess which component of a product is responsible for the observed effects. Unlike hard tribology, few studies have examined a range of single molecules roles within a system, especially in combination, where synergistic or anti-synergistic effects may be observed.

2.2.3.7 Progressing current literature

Despite the significant quantity of existing literature detailed above some areas remain highly promising for further advancement, without requiring a paradigm shift in technology, these primarily being production of low alcohol beers through novel yeast utilization and the instrumental physical characterisation through tribology. The usage of novel yeasts promises a potentially lower economic impact as well as streamlining production via the removal of additional processes. While tribology offers an avenue for investigating current products with a view to further understand the scientific basis of their flaws or advantages, currently literature of beer tribology is very limited, so this yields an opportunity to work on an early stage application with high industrial potential.

A further consideration to choice of target is the material requirements, dealcoholisation equipment utilizes several to hundreds of litres of beer per hour making its investigation in standard university laboratories challenging purely due to scale. While chemical composition

analysis of beer is a key area for quality control, the very significant existing literature would require a major shift in technology to provide a notable contribution to the existing field.

For these reasons, this work concentrates on the tribology and tribo-rheology of existing products and model systems, to further the understanding of the underlying basis of lubrication in beer rather than attempting to produce novel products.

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Chapter 3- Tribo-rheology of alcoholic and non-alcoholic beer

This chapter was published as:

Holt , T., & Mills, T. (2023). Tribo-rheology of alcoholic and non-alcoholic beer. *Journal of the Institute of Brewing*, *129*(3), 164–175. https://doi.org/10.58430/jib.v129i3.31

Thomas Holt: methodology, validation, formal analysis, writing (original draft, review and editing).

Tom Mills: methodology, writing (review and editing), supervision, funding acquisition.

Tribo-rheology of alcoholic and non-alcoholic beer

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Abstract

The analysis of mouthfeel is an important but challenging area for objective study. The common use of human tasting panels presents issues of comparability between studies and ethical challenges given the alcohol content of standard beer products. This article demonstrates analysis of a range of different commercially available beer products, representing seven distinct beer styles using tribo-rheology to demonstrate stylistic lubrication differences as well as differences observed within examples of the same style, featuring low and no alcohol beers compared to full strength examples. This method generated Stribeck curves which can differentiate between similar products. The work presented here also attempts to examine some possible causes for the observed differences, using ethanol, maltose, maltodextrin and salt contents. This work is able to demonstrate statistically significant differences in lubrication behaviour between low/no alcohol products and their standard strength alternatives from the same producer and present discussion of molecular detail of the observed effects.

Key words

Tribology; Tribo-rheology; low-alcohol; mouthfeel; beer

Introduction

Low and no alcohol beers represent a relatively small but growing market. The increasing consumption of no and low alcohol beers has, however, highlighted perceived historical quality issues thought to be present from the production methods which may deter purchase of modern products. Progress with methods of production of these products has aimed to remedy the defects identified within the early generation of low and no alcohol beers primarily the possible defects are categorized into volatile flavour, non-volatile flavour and mouthfeel.

Production methods for low and no alcohol beers have changed drastically since their introduction, very early methods simply used heating of the product to boil off ethanol, this process produces a highly undesirable product characteristic with significant loss of aroma molecules and oxidative damage to remaining compounds (Sohrabvandi et al. 2010). Modern production methods are generally characterised as alcohol removal or brewing at low alcohol. Within the ethanol removal strategies several options are commercially and experimentally available, these largely group into thermal methods and membrane-based methods.

While brewing based methods tend to be categorised based on altered condition brewing or non-standard yeast utilization (Bellut and Arendt 2019). The resulting products of all four major categories are expected to show different advantages and disadvantage (Rettberg et al. 2022). Of course, combinations of methods are also possible on the same product, or

potentially the final product being a mixture of two differently produced beers formulated together to produce a desirable result.

The true quantification of flavour is, however, challenging. Generally, gas chromatography with mass spectrometry is conducted on samples of beers to assess quality (Charry-Parra et al. 2011). Although this method is only capable of natively measuring volatile substances, it is commonly used to demonstrate quality in low alcohol beers, primarily in terms of volatile organic aroma compounds related to aroma and flavour. This choice is due to the historical methodologies of ethanol removal, which commonly involved heating (Brányik et al. 2012), as those volatile flavour molecules are the first to be lost when heated even with careful control. Gas chromatography is not the only methodology used in assessment of beers, liquid chromatography with mass spectrometry, often tandem mass spectrometry is also used, primarily for non-volatile organic acids, saccharides and other relevant molecules (Araújo et al. 2005) which are known to be important in taste, particularly sweetness, sourness and bitterness. Although gas chromatography is capable of measuring many of these, it requires significant processing and derivatization reactions, adding complexity to analysis compared to liquid chromatography (Otter and Taylor 1967).

Still more challenging than measuring absolute quantities of molecules but still key for beverage quality perception is mouthfeel, as mouthfeel is subjective and oral processing has been shown to have high individual variability (Hiiemae and Palmer 2003). The expected defects in mouthfeel vary, depending on the method of dealcoholizing or low alcohol brewing, classical low initial gravity mashing yields a wort low in fermentable sugar and thus a lower alcohol content. This leads to a lower final gravity as residual sugar is limited, final

gravity has been shown to strongly correlate with fullness in mouthfeel (Langstaff et al. 1991) and a low starting gravity beer would be expected to yield a thinner mouthfeel. Alternatively incomplete fermentation of a standard gravity wort could be utilized, this yields an unfinished fermentation process and is likely to induce significant flavour defects (Perpète and Collin 1999). These suboptimal or limited fermentation beers are also seen to exhibit high final gravity due to incomplete attenuation yielding a positive mouthfeel, but overly sweet taste with low levels of volatile aroma molecules (Perpète and Collin 2000).

The exact contributors to mouthfeel properties are less well defined than volatile aromatic molecules individual contributions to aroma but a range of different molecules are expected to be relevant. Early work to defined mouthfeel properties distributed perceptions into carbonation related, fullness and after feel (Langstaff and Lewis 1993), this was hoped to make it easier to describe the exact nature of the oral properties of a product. The molecular contributors to these properties were from a wide range of chemical classes and the reasoning for their contribution varied. For example, chloride ions were predicted to increase perception of mouthfeel indirectly, by initiating α-amylase production and chloride has been shown to positively correlate with perceived fullness (Langstaff et al. 1991). This is not an effect that can be measured using currently available instrumentation, as there is no capacity to simulate this release of enzymes although inorganic salts may have their own friction reducing effect in tribology experiments.

Classically dextrin concentration has been considered a major factor in mouthfeel perception (Langstaff and Lewis 1993) although more recently the ratio of lengths of saccharide polymer has been shown to have a significant role beyond purely the total concentration (Krebs et al.

2019). Using highly skilled panellists, it was possible to differentiate changes in mouthfeel beyond merely full or not, providing a more useful distinction for process and product adjustments (Krebs et al. 2019).

Ethanol concentration is frequently discussed as a major positive contributor to perceived mouthfeel, it is however, seen to show a complex relationship with viscosity when dissolved in water, (Khattab et al. 2012) linked to water chain formation and interruption (Ageno and Frontali 1967). Although more recent work looking at purely altered ethanol concentration in the same beer showed a complex interaction beyond higher levels being more positively received, highlighting individual perceptive differences to be a key factor (Ramsey et al. 2018).

Recently, tasting panel descriptions and scores have been compared to measured concentrations of molecules (Agorastos et al. 2023), this yielded a strong (0.84) correlation between iso-α-acid levels and bitterness, while polyphenol content was more weakly (0.59) correlated with drying (Agorastos et al. 2023). Iso-α-acids are expected to contribute significantly to bitterness (Caballero et al. 2012) but polyphenols are known to be the major contributor for drying/astringency in wine products (Laguna and Sarkar 2017), suggesting beer has significantly different behaviours to wines. Furthermore, it was also noted that ethanol concentration is not a major contributor to mouthfeel, as it was not correlated with attributes other than burning sensation. It has been previously observed that lower molecular weight sugars did not noticeably contribute to coating sensation (Agorastos et al. 2023). These correlations suggest that with human tasting panels directly attributing a single molecule or class of molecules to a descriptor is variably successful in the context of beer.

Human factors are known to vary widely, measured values for tongue movement vary from 2.10 to 32.43 mm/s across 165 individuals with the highest recorded being 305.67 mm/s (Hiiemae and Palmer 2003). This very wide range of speed is expected to result in very different lubrication properties and mouthfeel (Sarkar and Krop 2019) even when presented with the same product. Additionally, the force applied between hard palate and the tongue varies between individuals and based on stages of swallowing, ranging from 0.01-90 Newtons (Prinz et al. 2007), it was also observed that force varied significantly depending on exact location on the tongue.

Classically, analysis of beer was conducted by trained panels using predefined descriptors (Langstaff and Lewis 1993) which more recently have been compared and correlated with quantitative measurements from tribometers (Fox et al. 2021). However, the exact relationship between lubrication and mouthfeel is difficult to define and descriptions from participants vary depending on the substance being measured (Laguna et al. 2017; Batchelor et al. 2015).

Lubrication properties gathered by tribometer can be used to assess predicted oral properties of various liquid products (Batchelor et al. 2015; Cai et al. 2017; Godoi et al. 2017; Mills et al. 2013) as well as solids/semi solids (Samaroo et al. 2017; Ningtyas et al. 2019) and has specifically been used to measure beer (Fox et al. 2021) and wine (Laguna and Sarkar 2017) characteristics. The choice of surfaces is of key importance in tribology-based techniques and presents a dilemma for researchers as reproducibility is contrasted with relevance to biological systems. Of course, the most accurate to life system would be a hard

palate and tongue system. To the author's knowledge no one has attempted this complete measurement apparatus, but animal tongues have been used as a soft surface with a more standard moving surface (Ranc et al. 2006). Aside from ethical concerns, biological materials tend to be highly variable between organisms of the same species, let alone other genus, this makes comparisons between pig or other animal tongues and human ones challenging. As such, most studies opt for an artificial surface; most commonly polydimethylsiloxane (PDMS) is utilised (Laguna et al. 2017), although other silicone elastomers have been successful (Mills et al. 2013) as well as roughened tape (Godoi et al. 2017).

Recently, the use of dedicated tribology instruments has been expanded to include rheometers with tribology attachments, tribo-rheology. Which functions in a very similar manner, measuring the friction between two surfaces in the presence of a lubricant, but, due to making use of a rheometer very accurate sliding speeds are possible, as are slower sliding speeds. It also represents a potential cost and space saving as the instrument is capable of both standard rheology as well as tribology meaning a single instrument has dual functionality. Tribo-rheology is a newer technique and is less commonly used, so relatively little prior literature is available of specific systems using this technology. This system presents a method for analysing beer products to assist in quality comparisons of low and no alcohol beverages with their standard alcohol containing competitors using tribo-rheology.

Materials

Water for HPLC gradient analysis (Fisher Scientific), ethanol for HPLC (Fisher Scientific), sodium chloride analytical reagent grade (Fisher Scientific), maltose monohydrate analytical

reagent grade (Fisher Scientific) and maltodextrin 4-7 dextrose equivalent (Average 6.5 DE) (Sigma Aldrich), were used to create model systems. SYLGARD 184 elastomer kit (Dow Corning) was used to fabricate tribology surfaces. Commercial beer samples were purchased from a local supermarket and measured immediately after opening, all samples were from glass bottles. 0.22 µm poly-ethersulfone syringe filters (SLS) were used to remove any particulates from model test samples, beers were not filtered, but were allowed to settle for 48 hours before opening and use.

Instrumentation

Discovery hybrid rheometer HR-1 (TA Instruments) with 3 balls on plate top geometry (aluminum) (TA Instruments). Bottom sample holder was a locally produced 3D printed resin cup (STL file included in supplementary information). The axial force was fixed at 1 N (+/- 0.1 N).

A Melter Toledo handheld density meter Densito (accuracy +/- 1 g/mL) was used for specific gravity measurements based on an average of three measurements per sample.

Table 1. Reported ABV values, measured specific gravity

(N=3), and abbreviation codes for the tested samples.

Style	ABV	SG kg/m^3	Abbreviation
IPA	0	1008.2	IPA0
IPA	5	1005.7	IPA5
Amber ale	0	1013.1	AA0
Amber ale	4.3	1008.7	AA5
Lager	0	1016.3	LA0
Lager	4.6	1004.6	LA46
Lager	0.5	1015.0	LA05
Pale ale	0.5	1024.8	PA05
Pale ale	4.3	1007.0	PA43
Milk stout	0.5	1028.3	MS05
Milk stout	4.3	1016.4	MS43
German Wheat beer	5.3	1007.0	GWB53
German Wheat beer	0	1017.4	GWB0
Citrus pale ale	0.5	1013.9	CPA05
Citrus pale ale	4.5	1005.1	CPA45

Methods

Tribology measurements

Tribology was conducted using a TA instruments Discovery Hybrid Rheometer with 3 Balls on Plate attachment, this geometry consists of three ¼ inch diameter stainless steel hemispheres screwed into the flat plate attached to the main shaft with an aluminium spring beam coupling. Torque is measured while a constant axial force is maintained from the tribology attachment (1 N) and sliding speed is varied between 0.15 and 150 mm/s with 10 data points recorded per decade, temperature was maintained at 20 ^{o}C for all experiments, which were performed in triplicate using new PDMS surfaces for each replicate. Torque is then used to calculate friction coefficient designated μ, by the Equation: (Equation 1)

$$\mu = M \div dFN$$

Whereby M is torque (Nm), d is arm length (0.015 m) and FN designates the normal force (N).

Statistical analysis

Analysis was performed using Microsoft Excel 16 with Analysis ToolPak, one tailed t tests were conducted and P values of <0.05 were considered significantly different for the purposes of this work.

PDMS production and conditioning

PDMS disks were produced from SYLGARD 184 elastomer kits by mixing Part A 10:1 with part B (w/w), this was strongly mixed then degassed thoroughly, before being poured into resin moulds previously 3D printed yielding a depth of 4 mm (~4g). This was then cured at 100 ^{o}C for 35 minutes as per manufacturers recommendations, disks were sonicated with deionized water before use and only used for one measurement before being replaced.

Results and Discussion

Stribeck curves were generated for a range of commercially available beers of several styles, including low and no alcohol examples, this was conducted using a hybrid rheometer (see materials and methods). Stribeck Curves were compared to values obtained from deionized water), for ease of interpretation data is presented as style specific comparisons initially. Figure 3a shows the observed friction for two India pale ale style beers, both from the same brewery, with declared ABV of 0.0 (IPA0) and 5.0 (IPA5). From this data a clear difference is observed between all three samples, whereby the 5% ABV beer demonstrates a lower level of friction at all but the highest speeds, continuing to be statistically significant even at the

highest test speed (p=0.04) when compared to water. The 0% product is less distinct from pure water, as is seen by significant differences only being observed in friction between 0.6 and 75 mm/s sliding speeds. Low lubricity is a known feature of some low and no alcohol beers and is demonstrated by the differences observed in this comparison.

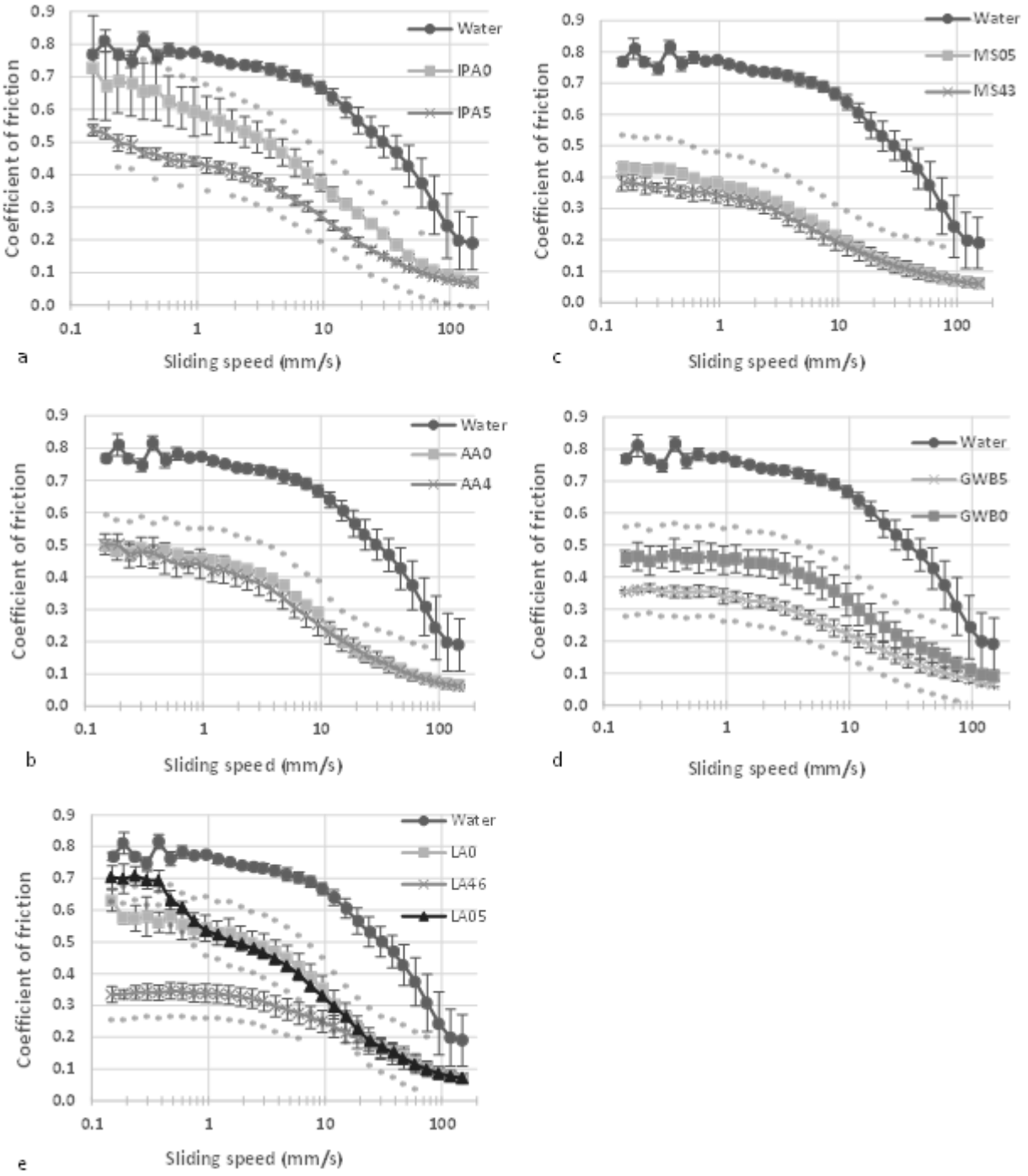

Figure 3. Stribeck curves generated from 3 ball on plate tribo-rheology on PDMS surface with water, IPA0 and IPA5 (a); AA0 and AA4 (b), MS05 and MS43 (c), GWB0 and GWB5 (d), LA0, LA46 and LA05 (e) as lubricants (n=3). Star markings denote significant difference from water (p=<0.05 in one tailed T test).

In contrast, other tested beers did not show such differences, the amber ales (AA0 and AA5) tested and shown in Figure 3b do not demonstrate a statistically relevant difference at any tested speed but are both distinct from water at all speeds below 75 mm/s. This similarity demonstrates a successful matching of lubricity between the two products from the same brewery in this instance. In similar fashion to the amber ale, the two milk stouts that were analysed exhibited much more similar lubricity than the two IPAs, despite the stouts being from different breweries. Figure 3c demonstrates the Stribeck curves obtained for these products, this style is expected to contain a high residual sugar content obtained by the addition of lactose prior to fermentation. To investigate this specific gravity readings were taken of all samples and are displayed in Figure 4. The expected higher final gravity was clearly visible in the 4.3% ABV example, where specific gravity was measured at 1.0164, while the next highest alcoholic beer was measured at 1.0087 and the average alcoholic beer being 1.0078. The difference is less apparent, however, in the low and no alcohol beers, where the 0.5% ABV milk stout is measured at 1.0283 but the average for this class is 1.0172. The relatively high final gravity of the low alcohol beers is expected and is generally a by-product of their limited fermentation process (Sohrabvandi et al. 2010) or from post fermentation additions of saccharides.

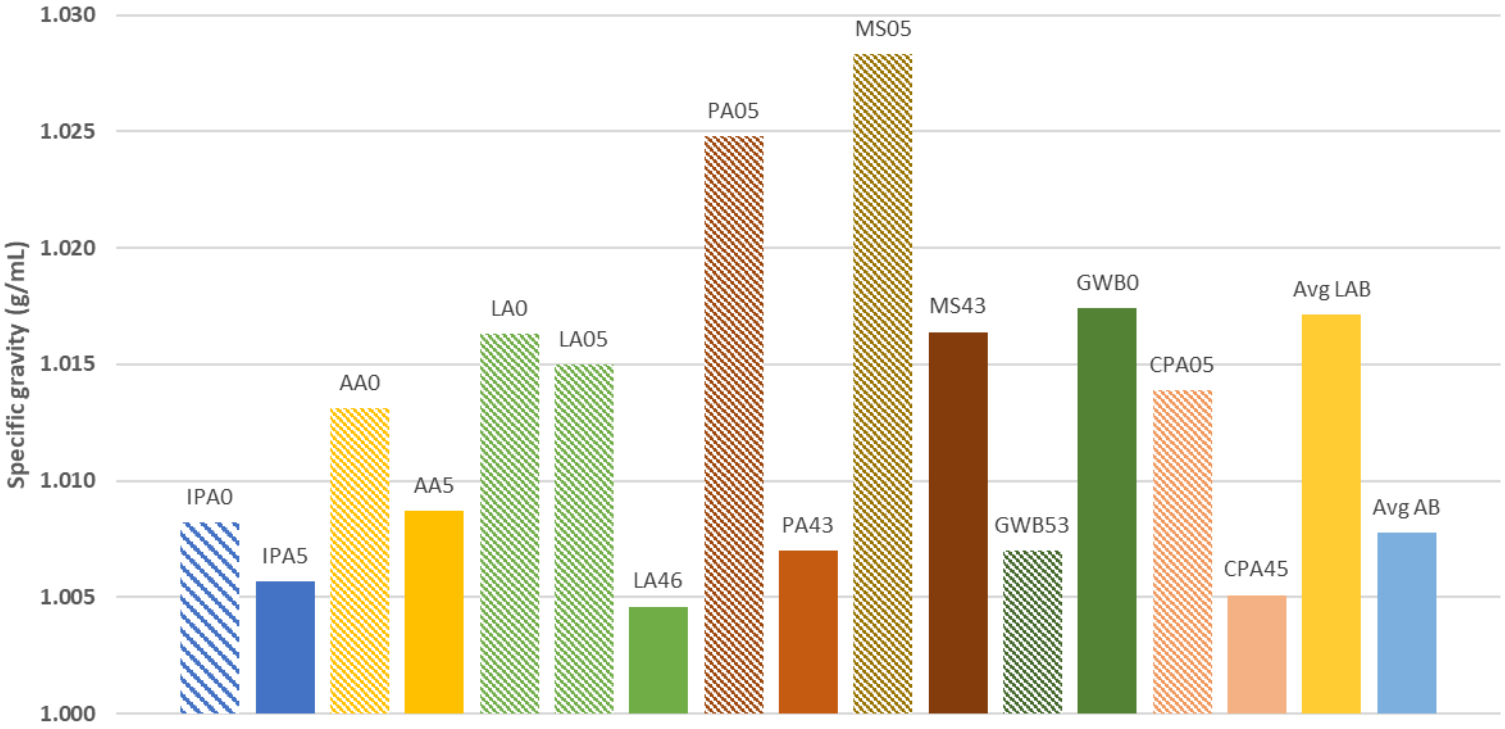

Figure 4. Specific gravity measurements of beers used in this study with the mean for all low alcohol beers (avg LAB) and the mean for alcoholic beers (avg AB). Data collected using a Densito densitometer (+/- 0.001 g/mL).

Another style known for its mouth feel is German Wheat beer and, tribo-rheology was conducted on two samples, one reported at 5.3% ABV the other 0.5% ABV, both are from the same brewery. Figure 3d shows the Stribeck curves obtained from these. From this data it is shown that significantly higher lubrication is observed from the alcoholic beverage at almost all sliding speeds. The level of lubrication shown by the low alcohol beer is also significant, yielding a statistically relevant difference when compared to water at all speeds below 75 mm/s, this is likely explained by the above average specific gravity of this product (1.0174) and labelled 53 grams carbohydrate per litre (Figure 4).

Lager style beers are generally expected to have a milder taste profile with reduced bitterness and limited hop impact (Furukawa Suárez et al. 2011), when compared to ales. This can be advantageous in terms of producing low alcohol versions as the more subtle flavour is not as adversely affected by limited fermentation or ethanol removal strategies. As such, a standard strength European lager and the same brewers 0.0% ABV products were compared, Figure 3e shows the Stribeck curves. This again shows a significant difference between the 4.6% abv and the alcohol free, in this case, primarily in the lower speed region which is expected to have greater relevance to oral processing, with 10.34 mm/s being reported as the mean speed of movement during swallowing of liquids (Hiiemae and Palmer 2003) although variation and range was significant between individuals. Further to this analysis a second lager style beer was obtained, produced by a different brewery for comparison, this comparison shows a similar profile to that seen from LA0, except at measured speeds of 0.37-0.18 mm/s where a significant difference is observed between LA0 and LA05. The difference is, however, that the 0.5% ABV beverage shows increased traction,

specifically in this speed range, this is generally not expected, as ethanol has been shown to produce a lubricating effect (Mills et al. 2013).

The trends demonstrated in Figure 3 differs from previous work conducted by Fox et al primarily in that friction curves for no alcohol beers were previously seen to exhibit lower friction factors than the alcohol containing versions of the same product (Fox et al. 2021). Whereas this present study shows none of the studied low and no alcohol products to have lower friction coefficients than the alcohol containing versions. As none of the beverages measured in Fox et al were used for this study so no direct comparison between methods is possible, it is also noted that Fox et al made use of a glass ball surface with PDMS pegs (Fox et al. 2021). This contrasts to the stainless steel 3 ball on plate with flat PDMS discs employed here, it is possible that observed differences are due to the different surface chemistry of glass vs stainless steel and or the different application of force seen in a single ball on pegs apparatus versus 3 balls on a disk. This does suggest that equipment choice may have an important role in measurements and suggests some standardization on methodology or calculation of conversion factors could benefit the field.

To investigate the source of this variation found within our own data, solutions of various substances known to exist in beers were produced and analysed by the same process as the beverages. Ethanol was chosen initially as this is the most obvious change between the products, Figure 5a shows the Stribeck curves obtained from a series of ethanol concentrations. Interestingly, 0.5% ethanol is seen to significantly increase the friction seen at lower speeds (<7.5 mm/s), this observation suggests there may be a threshold where very low concentrations of lubricating substances are in fact less lubricating than the absence of

them. This observation is likely related to incomplete tribofilm formation due to insufficient concentration of suitable molecule, this specific concentration also has a direct relevance to this study as many low alcohol beers are reported at 0.5% ABV. This data therefore suggests a possible cause for the difference seen in between LA0 and LA05. With the 0.5% ethanol increasing friction. While the zone of increased friction seen between the two beers is not identical to that of 0.5% ethanol and water it shares some overlap suggesting at least some possible link.

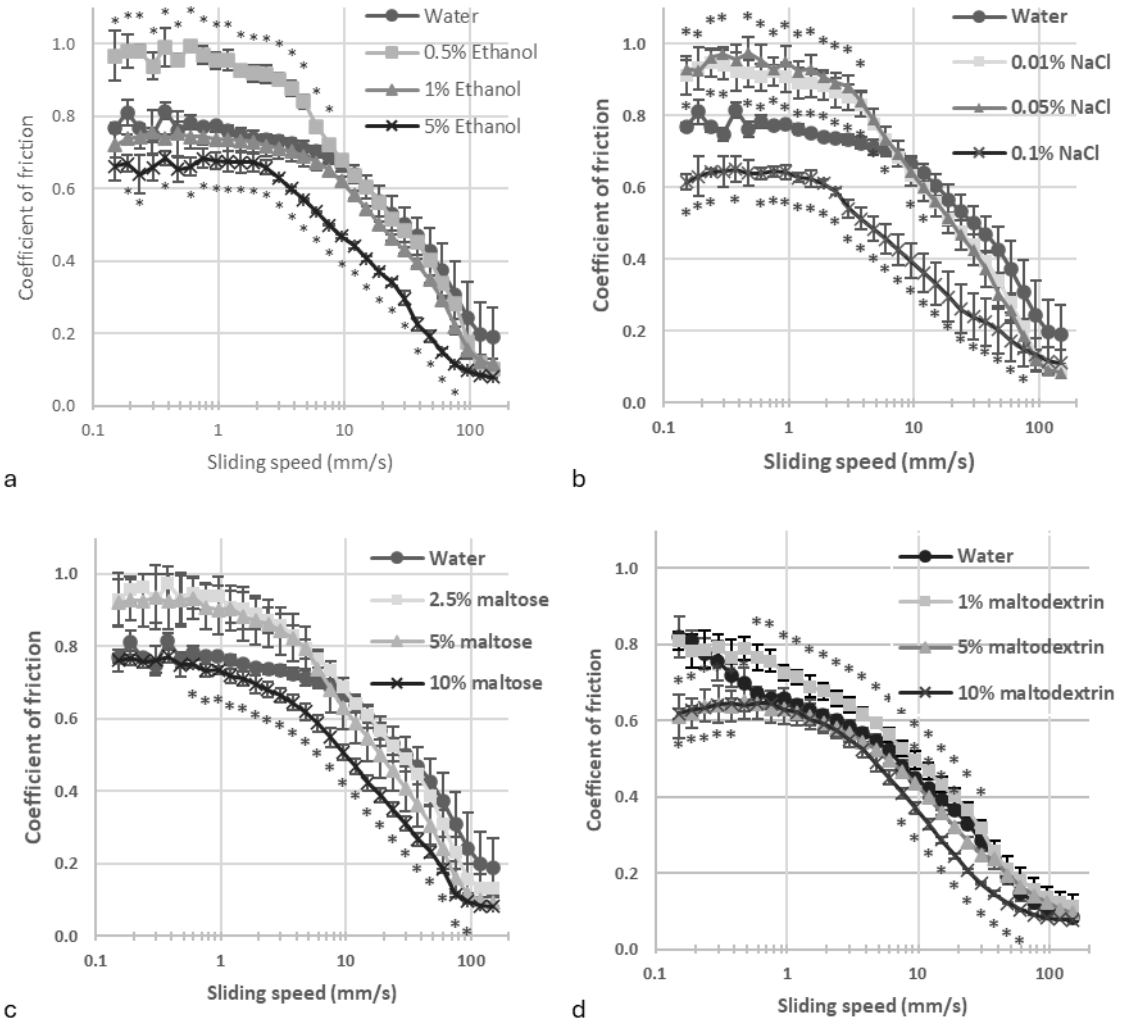

Figure 5. Stribeck curves generated from 3 ball on plate tribo-rheology on PDMS surface with water, and various concentrations of; ethanol (v/v) (a), maltose (w/v) (b), sodium chloride (w/v) (c) and maltodextrin (w/v) (d) in water solutions as lubricants (n=3). Star markings denote significant difference from water (p=<0.05 in one tailed T test).

Once a concentration of 1% ethanol is reached almost no significant difference in lubrication is observed from water, this indicates the threshold for neutral effect is between 0.5 and 1% for this lubricant. By the 5% ethanol level however, significant lubrication differences are observed at almost all speeds compared to water. This level also potentially suggests an interesting although most likely unintentional outcome of brewing, where by 5% ABV is on the lower end of ethanol levels that yield a significant difference in friction and represents a common ethanol level for brewed alcoholic beverages. This hypothesis would require significantly deeper investigation, particularly in more complicated systems but could represent an interesting avenue of investigation for the evolution of a brewing historically. Figure 6 shows a proposed schematic view of the molecular organisation for ethanol based tribofilms in this system, here the less polar carbon chain of the ethanol molecules interacts with the methyl groups present in the PDMS surface, while the polar hydroxyl groups interact with the chromium oxide layer present on the stainless-steel upper geometry.

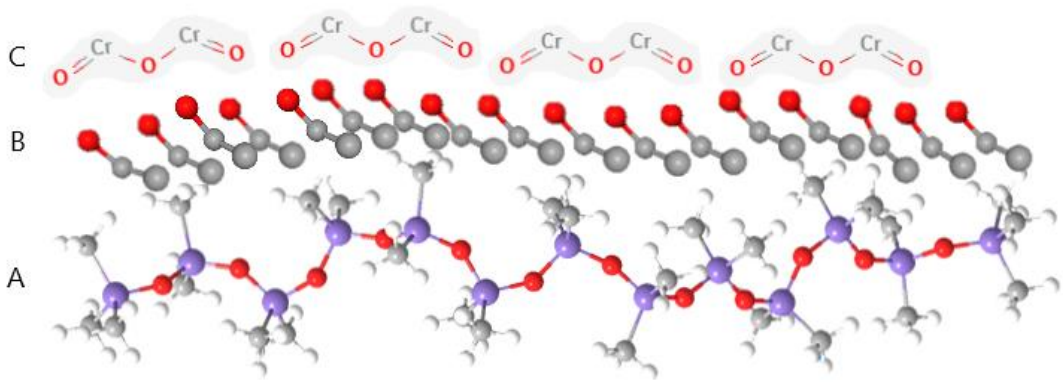

Figure 6. Schematic of the proposed interactions of ethanol with PDMS and stainless steel within the tribo-system. (A) shows the PDMS polymeric chain (length is not representative of those expected but for visual representation), (B) demonstrates the proposed orientation of ethanol molecules and (C) represents the chromium oxide layer present on stainless steel.

Following on from the observations with ethanol solutions, maltose was selected to act as a model for all residual sugars for this study, clearly the mixture of saccharides seen in products is far more diverse (Otter and Taylor 1967), however maltose was selected as a representative fermentable wort sugar. Stribeck curves were obtained for several concentrations of maltose (Figure 5b), this data shows an interesting pattern similar in some ways to ethanol, whereby at low concentrations and low speeds traction is increased. Although for maltose the concentrations required are significantly higher (0.5% vs 2.5-5%) the change is statistically significant at more points of speed.

Previous work has demonstrated a role for larger chain polymeric saccharides in sensory perception of beers (Krebs et al. 2019), as such maltodextrin (4-7 DE) was also tested (Figure 5d). A similar profile is observed with these larger saccharide chains as was seen with maltose; whereby the lowest concentration demonstrates significantly lower lubricity than water, although in maltodextrins case the sliding speed range for significant difference is faster, covering a significant portion of the tested speeds. Interestingly 5% and 10% solutions show similar behaviour at lower speeds but are significantly different at higher speeds, where 5% solutions are not significantly different from water while 10% is. This is consistent with data from previous studies where 50 g/L was found to be the lowest concentration with any significant effect on mouthfeel (Krebs et al. 2019).

To further investigate these observed effect, sodium chloride solutions were analysed at a range of concentrations, as simple inorganic salts have previously been shown to demonstrate lubrication behaviour in solutions (Mills et al) and this represented a possible cause for some of the observed changes. Figure 5c shows the results obtained from this

analysis, sodium chloride is used as a substitute for total mineral content as the actual inorganic make up of beverages varies significantly, based on the local water used or remineralization of purified water (Krennhuber et al. 2016). Similarly, the total salinity is seen to vary significantly, as such the samples for this work were not intended to replicate any specific product or style and just represent a simple model for inorganic content of beers. From the data generated, it is again seen that at low concentrations lubricants can increase friction. Total mineral content in beer was recorded as 363-700 mg/L (Krennhuber et al. 2016) including alcoholic and non-alcoholic beers, suggesting the levels measured here are within those measured in commercial products.

The initially obvious suggestion as to why the different molecules had varied thresholds to act as lubricants is that, due to molecular mass differences there are similar levels of molecules present. The molar concentrations were calculated and the Stribeck curves replotted as Mol/L concentrations (Figure 7). From this, it is apparent that sodium chloride provides greater lubrication per mol than either other tested molecule while ethanol and maltose are somewhat similar at lower concentrations (0.0857 Mol/L and 0.0694 Mol/L) but begin to diverge when higher levels are considered. The observed increases in friction are likely from boundary chemical films related to elastohydrodynamic lubrication where in the lubrication films formation is dependent on viscosity as well as chemical properties of both surfaces and the lubricant (Hsu).

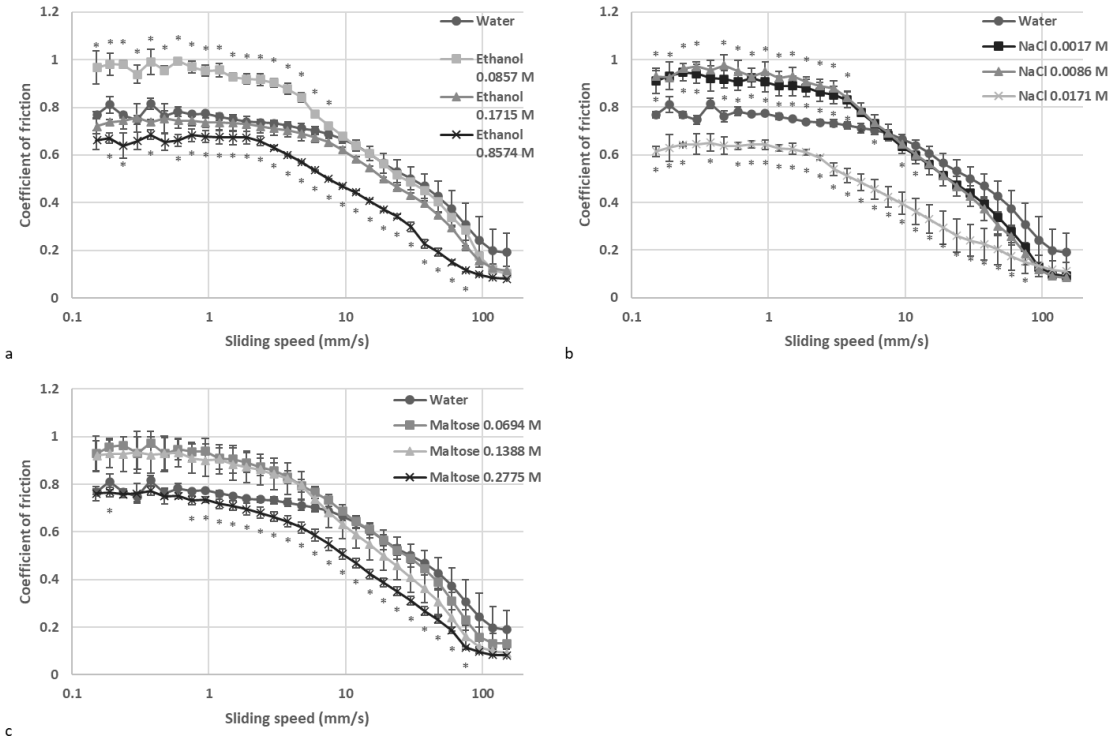

Figure 7. Stribeck curves generated from 3 ball on plate tribo-rheology on PDMS surface with water, and various concentrations of ethanol (a), sodium chloride (b) and maltose (c) in water solutions M/L as lubricants (n=3). Star markings denote significant difference from water (p=<0.05 in one tailed T test).

The concept of monolayer film lubrication is well founded but is primarily applied to large fatty acid, siloxanes and thiols, classically in Langmuir-Blogett films. These films are only seen to behave as solids when the molecular spacing is equal to or smaller than the size of the film forming molecule, when not under these conditions the film behaves as a liquid monolayer rather than a solid one. These liquid layers are more resistant to failure as the molecules are able to move under stress without causing total disruption of the system but only when under relatively light stress levels (Hsu 2004). This natural flexibility along with the ability to self-repair by diffusing back into the monolayer allows molecules to provide physical lubrication for surfaces. The presence of a range of differently sized molecules allows for more easy formation of these layers by tessellation of the different sizes, shapes and

polarities to produce the most thermodynamically stable result. A key consideration with these mixed monolayers is compatibility of molecules, it is expected that some functional groups will reduce binding and tessellation of certain other classes (Hsu 2004), this is important in complex systems such as beer, where many different types of molecules are present and competing for binding spaces. It has been demonstrated that competitive binding from poorly compatible molecules produces inferior lubrication effects than single component systems dependent on the compatibility of the molecules used (Nakayama and Studt 1991). With the presence of many different molecules the formation of crowded Langmuir-Blogett films may become more likely as the gaps between bound molecules can be filled creating a more uniformly covered surface although this will also be highly dependent on molecule compatibility and relative concentrations. These binary interactions at surface interfaces are difficult to predict and may be concentration independent if binding is blocked or inhibited by the other molecules present.

The application of Langmuir-Blogett film theory to heterogeneous wear surface systems i.e., where two different materials are abraded against one another, is less commonly observed as much of this work is applied to metal-metal based wear interactions. Oral tribology requires a softer surface be used for one of the tribopairs, this allows scope for substances to form lubricating surfaces on one of the pair but not the other. This pairing-based system also brings the possibility of two entirely different monolayers, one adsorbed to the metal and the other to the PDMS or other soft surface, further complicating the study of complex mixtures. Here you may be measuring analyte-analyte interactions as the two different monolayers abrade and interact with each other, or form more complex chains from the

original surfaces, producing effects unique to that mixture of lubricants and those tribopairs only visible at speeds where elastohydrodynamic effects do not dominate.

Conclusions

Tribo-rheology provides an effective methodology for measuring lubrication properties of commercial beer and allows for investigation into possible causes of observed differences. It was able to demonstrate differences in lubrication behaviour between Indian pale ales, German wheat beers and one lager beer with different alcohol levels, which is likely due to the loss of lubrication performance provided by the ethanol content. The method was also able to demonstrate that measured amber ales, milk stouts and two lager beers closely match their standard strength suggesting some compensation for the lack of ethanol as a lubricant has occurred from the different overall formulation. This method presents a mechanism for more complex artificial systems to be examined to elucidate causes of physical property differences in products as well as functionality in validating experimental brewing techniques in attempting to mimic specific desirable lubrication properties.

Author Contributions (CRediT)

Thomas Holt (ii methodology, iv validation, V formal analysis, IX writing original draft, X writing-review and editing.

Tom Mills (ii methodology, X writing-review and editing, xii supervision, xiv funding acquisition).

Acknowledgements

The authors would like to thank David Beverly and Frank Lynch for assistance in reviewing the manuscript and general support with the work.

Conflicts of interest.

The authors declare there are no conflicts of interest.

Funding statement

Funding was provided by the University of Birmingham Adrian Brown Scholarship in brewing research.

Supplementary information

STL file for 3D printed bottom geometry.

.STL1.

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Chapter 4- A microstructure approach to tribo-rheology of beer by molecular composition

This chapter was submitted as:

Holt , T., & Mills, T. (Awaiting peer review 12/12/23). A microstructure approach to tribo-rheology of beer by molecular composition. *Journal of the Institute of Brewing Manuscript ID JIB20231535*

Thomas Holt: methodology, validation, formal analysis, writing (original draft, review and editing).

Tom Mills: methodology, writing (review and editing), supervision, funding acquisition.

A microstructure approach to tribo-rheology of beer by molecular composition

Key words: Tribo-rheology, Beer, Microstructure, Mouthfeel, Authors

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Abstract

Why was the work done: To investigate the potential role of previously understudied molecules in mouthfeel of beer and the effect of the presence of absence of ethanol on these interactions.

How was the work done: Solutions of selected molecules previously quantified within beers were selected and their tribo-rheological properties measured by generation of Stribeck curves, both alone in water and in 5% ethanol. Mixtures of these molecules were then studied, again with and without ethanol. Correlation analysis was then used to give insight into which, if any chemical properties could be useful predictors of tribological behaviour for molecules.

What are the main findings: The tribological behaviour of molecules was not predictable by concentration, molecular mass, topographic polar surface area, pKa or LogP. As low concentration small molecules were shown to greatly effect the properties of higher concentration molecules making mixtures of even two different molecules challenging to predict. This indicates a role in lubrication for low concentration volatile aroma compounds specifically, as acetoin especially was found to have significant effects on the behaviour of

systems. Ethanol was also found to interact unpredictably with molecules, not always yielding a reduction in measured friction.

Why is the work important: Mouthfeel is an often academically understudied property of beer, and the findings here indicate possible roles for often disregarded classes of molecules within this field. The simple systems experimented on here demonstrate a high level of instrumental sensitivity, suggesting this method could be readily applied to more complete beer systems to investigate how brew house, fermentation or post fermentation changes may alter the mouthfeel of products.

Introduction

Tribo-rheology is an emerging approach to the approximation of mouth feel of products from several major sectors, with previous work completed on oral medications (Batchelor et al. 2015), dairy products (Godoi et al. 2017), fluid gels (Mills et al. 2013), wine (Laguna and Sarkar 2017) and beer (Fox et al. 2021; Holt and Mills 2023). The technique involves the running of a harder surface against a softer one under downforce, while measuring the torque required to maintain the speed of sliding. The choice of surface is up to the given researcher, in food and beverage studies stainless steel or glass are commonly used as hard surfaces with soft surfaces most commonly produced from polydimethylsiloxane (PDMS) (Sarkar and Krop 2019). Although silicone elastomer (Mills et al. 2013) and roughened surgical tape has been utilized (Ningtyas et al. 2019), PDMS is more commonly selected.

The selection of surfaces is expected to have a significant influence on the lubricity observed, for example standard PDMS is hydrophobic (Mukhopadhyay 2007) and is known to strongly

adsorb small hydrophobic molecules from its environment with sufficient time (Mukhopadhyay 2007) as well as notable capacity to adsorptive proteins (Chumbimuni-Torres et al. 2011). While surgical tapes and silicones are available with many different properties depending on the exact product selected, as such their properties may be harder to standardize between studies unless the exact product is available. Due to the varied properties of these surfaces, it is expected that measured lubrication will differ significantly from lubricant interactions with the surface, even if an identical hard surface is used.

Also available to researchers is the ability to add saliva or a saliva analogue, Sarkar and Krop found 6 out of 13 studies they reviewed had attempted some form of saliva, real or artificial (Sarkar and Krop 2019). The inclusion of initial aqueous lubricant in the form of saliva does not just provide liquid to assist with dispersing the sample, depending on the nature of the saliva analogue it is likely to also contain a range of large and small molecules with intrinsic tribological properties as well as abilities to interact with and alter those of the sample (Morell et al. 2017). While this addition aids with replication of *in vivo* environments it also represents a significant complication to more simple systems, given the intrinsic properties of commonly used artificial saliva more minor effects from samples may be difficult to discern with the added complexity of the saliva analogue masking lower concentration or less effective lubricants. These minor effects could be lost when a more complex tribological system is attempted, meaning basic two component systems are not necessarily strictly less efficacious, but are harder to correlate with in-vivo perception.

The general approach used by most studies is to measure the properties of a finished commercial or experimental product, an alternative but less common approach is to test

specific molecules from a mixture or product of interest. This molecular approach is more frequently used in wine astringency studies (Wang et al. 2020; Laguna et al. 2017) where tannin is considered the primary cause of the perception of astringency, as well as in mechanical tribology where single lubricant additives are commonly tested in formulation research (Minami 2009; Gulzar et al. 2016). This molecular approach is not frequently used to isolate or investigate the individual role of many different molecules however, this work will investigate its potential in the identification of molecules with previously unrealised roles in the tribological behaviour of beer systems.

The basis for the lubrication properties of a given liquid is determined by the entrainment speed (Wen and Huang 2012), at higher speeds the hydrodynamic lubrication regime dominates, with the lubricant preventing any molecular contact between the two surfaces. This lubrication is characterised by low friction forces due to the absence of asperity contact by virtue of physical distancing between the surfaces (Hsu 2004). The lubricity is dependent on the bulk properties of the fluid, primarily viscosity as this determines the ability of the fluid to entrain between the two surfaces. As speed is reduced the hydrodynamic lubrication will begin to fail and the surfaces start to show some areas of contact, known as the mixed regime, here surface roughness is relevant to how thick the fluid layer must be to prevent the contact. For example, more uniform surfaces will not require such a depth of fluid to fully separate the structures of the surface and so prevent them contacting the other wear surface, while a roughened surface will require a greater depth of fluid to achieve the same effect. This is visible at both the macro and micro structural level, where fluid no longer prevents the interaction of the wear surfaces asperity contacts will cause an increase in friction forces (Hsu 2004). Here the molecular composition of the lubricant becomes relevant

above its bulk properties, allowing for adhered molecules to form a tribo-corrosive film surface, these temporary films provide a uniform but temporary structure for the contact (Thrush et al. 2021; Willermet et al. 1995). The tribofilms reduce friction forces due to their more uniform nature as well as reducing wear on the original material by providing a sacrificial surface which can be worn away. The temporary nature of these films is offset by regeneration, which can occur as long as some fluid is available to deposit more molecules to replace those which are worn away, the actual thickness of tribofilms varies significantly having been measured between 100 nm and 300 nm largely determined by contact pressure (Hsu 2004). The nature of attractions within these films is also highly varied, with Van der Wals, dipole and full chemical bonding possible (Ismail and Bagheri 2017; Lim et al. 2022; Hsu 2004). Due to the varied nature of bonding the physical strength of tribofilms is highly varied, based upon the molecular composition of both the original surface and the lubricant. When corrosion due to friction is greater than regeneration the film will begin to collapse, depending on the rate of corrosion this may be very rapid showing a significant increase in friction suddenly as the entire film system fails, or more gradual as it is worn down locally and eventually no cohesive structure remains.

Classically beers would be assessed by human tasting panels attempting to judge mouthfeel along with other factors such as bitterness, sweetness etc. (Harrison 1970; Meilgaard et al. 1979). This approach while effective given the similarity to the final assessor of quality is time consuming in terms of person hours spent, with guides and tasters being required to spend significant time on the task. This can represent a significant expenditure to ensure it is conducted properly, as limited numbers of, or similar demographics of participants may bias results (Stone and Sidel 2004). An ongoing challenge even for experienced tasting panel

members is to describe or quantify multiple parameters, for example separating acidity and mouthfeel can present problems as can very strong presentations of one attribute covering another.

Analytical instruments, however, are able to categorically quantify properties of a product. This grants the advantage of objective quantification, but especially with food products the absolute concentrations are not always perfectly correlated with human perception of the products (Ramsey et al. 2021). A major advantage of instrumental measurement is high throughput, as instruments do not require breaks or experience palate fatigue, they are able to run many test products within a short period. The primary downside of instrumentation is a high initial cost, although costs per hour are generally lower than an entire panel of tasters would be, they do require permanent space in a facility, electricity and possibly other consumables depending on the exact type of analysis.

Breaking complex biological products down into individual components is a challenge due to the sheer variety of molecules present in these samples. As such some selectivity much be applied when deciding which molecules to examine, this work includes a broad selection of molecules designed to cover the breadth of diversity found within beer, but also keep the study to a manageable scale. Molecules were selected to cover many chemical classes, mono-valent metal salts, di-valent metal salts, esters, organic acids (mono-valent, di and trivalent), simple alcohol, branched alcohol as well as di-saccharide, trisaccharide and polysaccharide. In addition, a commonly available model protein was utilized as a proxy for barley/wheat proteins found in beer.

Methods and Materials

Water HPLC plus grade (Sigma Aldrich), Ethanol absolute for HPLC (Fisher Scientific), sodium chloride analytical reagent grade >99.98% (Fisher Scientific), maltose monohydrate analytical reagent grade >98% (Fisher Scientific), raffinose >99% (Thermo Scientific), acetoin >95% (Alfa Aaesar), bovine serum albumin >96% (Sigma Aldrich), calcium chloride dihydrate analytical reagent grade >99% (Fisher Scientific), magnesium chloride anhydrous 99.8% (Acros Organics), potassium chloride >99% (Sigma Aldrich), 3-methyl-1-butanol >98% (Sigma Aldrich), acetic acid >99% (Sigma Aldrich), lactic acid 85% ACS grade (Sigma Aldrich), L (+) Aspartic acid >98% (Acros Organic), L-isoleucine >98% (Sigma Aldrich), L-(+)-lysine >97% (Tokyo Chemical Industries), L-phenylalanine >99% (Sigma Aldrich), valine, L-leucine >98% (Sigma Aldrich), L-serine >99% (Sigma Aldrich), maltodextrin 4-7 DE equivalent (6.5 dextrose equivalent mw ~3008) (Sigma Aldrich), succinic acid >98% (Fisher Scientific), citric acid >99% (Fisher Scientific), ethyl acetate analytical reagent grade (Fisher Chemical).

Table 2. List of molecules tested, with the name used within the text, IUPAC and CAS identifiers with the concentration utilised with the literature source for that information.

In text name	IUPAC	CAS	Concentration (mg/L)	Source
Acetoin	3-hydroxybutanone	513-86-0	14	(Ojala et al. 1994)
Ethyl acetate	Ethylethanoate	141-78-6	32	(Verstrepen et al. 2003)
Isoamyl alcohol	3-methyl-1-butanol	123-51-3	41	(Charry-Parra et al. 2011)
2-phenylethanol	2-phenylethanol	60-12-8	16	(Charry-Parra et al. 2011)
Citric acid	Citric acid	72-92-9	186	(Klopper et al. 1986)
Lactic acid	3-hydroxypropanoic acid	50-21-5	1362	(Klopper et al. 1986)
Succinic acid	Butanedioic acid	110-15-6	166	(Klopper et al. 1986)
Sodium Chloride	Sodium Chloride	7647-14-5	230	(Pohl 2008)
Potassium chloride	Potassium chloride	7447-40-7	1100	(Pohl 2008)
Calcium chloride	Calcium chloride	10043-52-4	140	(Pohl 2008)
Magnesium chloride	Magnesium chloride	7786-30-3	265	(Pohl 2008)
Leucine	(*S*)-2-Amino-4-methylpentanoic acid	61-90-5	159	(Fontana and Buiatti 2009)
Phenylalanine	*S*)-2-Amino-3-phenylpropionic acid	63-91-2	99	(Fontana and Buiatti 2009)
Aspartic acid	(*S*)-(+)-Aminosuccinic acid	56-84-8	82	(Fontana and Buiatti 2009)
Lysine	(*S*)-2,6-Diaminocaproic acid	56-87-1	80	(Fontana and Buiatti 2009)
Isoleucine	(2*S*,3*S*)-2-Amino-3-methylpentanoic acid	73-32-5	159	(Fontana and Buiatti 2009)
Serine	(*S*)-2-Amino-3-hydroxypropionic acid	56-45-1	63	(Fontana and Buiatti 2009)
Bovine Serum albumin (Protein total)	N/A	9048-46-8	5000	(Fontana and Buiatti 2009)

SYLGARD 184 elastomer kit (Dow Corning) was used to fabricate tribology surfaces as per manufacturer's instructions using 10:1 ratio of polymer to crosslinking agent (w/w).

Instrumentation

Discovery hybrid rheometer HR-1 (TA Instruments) with 3 balls on plate top geometry (aluminium) (TA Instruments). Bottom sample holder was a locally produced 3D printed resin cup (STL file included in supplementary information) supplemented with a PDMS disk, which was replaced between each of the 3 replicates. The axial force was fixed at 1 N (+/- 0.1 N) while sliding speed was varied from to 150 mm/s to 0.15 mm/s with 10 data points recorded per decade. This system was used to produce Stribeck curves for test samples, the data from which was then used to produce difference from solvent graphs by subtracting values obtained from the solvent system from samples.

Sample Production

All components except ethanol were produced weight per volume using grade A volumetric flasks (Fisher Scientific).

Ethanol was added volume per volume using grade A volumetric flasks (Fisher Scientific).

0.22 µm poly-ethersulfone syringe filters (SLS) were used to filter any particulates from the tested samples before testing. Concentrated stock solutions produced this way were stored at 4 °C before being diluted and equilibrated to room temperature, samples were then filtered again immediately before usage.

Statistical Analysis

Microsoft Excel version 2307 was utilized to conduct one sided T tests to ascertain where difference were significant, tests returning p values <0.05 were considered significant for this work.

Results and Discussion

Due to the varied nature of the compounds selected, even within their own chemical class, several chemical properties have been used here to assist in examining their effects. Briefly, these are pKa, logP, topographic polar surface area (TSPA) and molecular mass. pKa is a measure of a compounds likelihood to dissociate a proton and is derived from the negative log of the concentration of H^+ ions released (Sörensen 1909). This effectively accts as a measure of strength in organic acids and can have some relevance to other molecules in terms of protonation, within tribology this likelihood of dissociation is related to the binding opportunities it will present. Deprotonated molecules offer a fully charged surface, while still protonated molecules may be polar, but not formally charged, this will dramatically alter the binding potential of the molecule. This is especially relevant where mixtures of two acidic compounds are considered, as the one with a lower pKa will force the other to remain protonated (assuming pH is sufficient), reducing its opportunity to bind to charged surfaces. LogP is a measure of the solubility of a molecule in water vs 1-octanol, yielding a partition coefficient expressed as logP, this value is primarily used in pharmaceutical drug development as it has been found specific values yield increased blood-brain barrier crossing potential (Culler et al. 1996; Gratton et al. 2011). In a tribological context this value provides an idea of overall polarity, but for this work is of particular use due to the inclusion of ethanol, which would be expected to negatively effect the solubility of molecules with a low logP (indicating poor solubility in octanol and high solubility in water). This reduced solubility may increase deposition of such molecules upon surfaces if polar surfaces are available to shield the polar regions of binding molecules from less polar solvent phases.

Topographic polar surface area is a computationally derived measure of the surface composition of a molecule, it considers the volume of all polar atoms and their hydrogens to yield a value for use in assessing potential binding (Prasanna and Doerksen 2009). TPSA has an obvious application to tribology, in that it describes the surface of molecules more so than their overall nature, this is a key determinant of tribo-film formation potential and interior sections may not be accessible for interactions so their polarity is irrelevant in terms of film forming.

Previously pKa has been investigated in protic ionic liquids as a factor in friction reduction, particularly at high temperatures (far greater than relevant to oral tribology) (Hatsuda et al. 2020). Historically the usage of pKa in tribological interactions has been linked to surface wetting of acidic or basic compounds (Fowkes 1981), this wetting was related to polymers, such as those previously utilized in tribological studies and has been used to explain the weak interactions of inorganic molecules with polymers (Fowkes 1981). pKa has also been used to explain binding properties of phospholipids based on their polar side chains in the context of their tribological efficacy (Pawlak et al. 2016)

In contrast to pKa, to the authors knowledge logP has not been significantly investigated in terms of tribology before, although it may sometimes used to quantify the polarity of additives as a standard chemical property.

TPSA has however been used as a possible computationally predictive method for tribological function in terms of monolayer formation using database information and

predictive modelling (Summers et al. 2020). This predictive capacity is however, highly surface dependent and slight changes in surface chemistry may yield different results.

Single molecule systems

Initially the single molecule solutions in water and single molecule solutions in 5% ethanol were examined to achieve a baseline for predicting lubricity in more complex environments. As the solvent for all experiments is either pure water or 5% ethanol in water these were used as baselines to quantify any alteration in lubrication properties. Results are presented as solvent subtracted showing only the difference in friction exhibited by the molecule compared to the solvent system alone (either water or 5% ethanol in water).

Given the large number of combinations measured during this study, molecules have been broadly grouped based on their effect on lubricity to aid with the description and interpretation and some combinations have been omitted from the main text.

Volatile Aromatic Systems

Traditionally volatile aromatic compounds are not considered significant contributors to mouthfeel due to their relatively low concentrations, primarily being under 100 mg/L except in extreme cases. However they have been seen to alter perceptions of mouthfeel (Symoneaux et al. 2015). Figure 8 shows the results of tribo-rheology performed on ethyl acetate (EtOAc), isoamyl alcohol (ISA) and acetoin in both pure water systems (8A) and 5% ethanol (8B). The demonstration of significantly higher friction at speeds under 7.5 mm/s in ethyl acetate/water and isoamyl alcohol/water systems is interesting, partially due to the

marked similarity between them. While representing different classes of volatile organic molecule, both have very similar molecular mass (88.11 vs 88.15) and present similar logP values (0.73 vs 1.16) and at higher sliding speeds follow a similar pattern. This then diverges as speed is reduced, finally showing markedly different values, but a similar overall shape to the speed vs friction relationship. Also of interest is acetoin, which shares a molecular mass of 88.11 but is present at much lower levels in commercial beer, due to its often undesirable taste (Haukeli and Lie 1975). At a level considered below taste threshold but also representative of a commercial beer product it produces a non-significant ($p>0.05$) effect at any speed. This is then however contrasted markedly with its behaviour upon addition of ethanol, here a significant increase in friction at speeds below 1 mm/s is observed. This suggests acetoin is able to cause some disruption to lubrication properties afforded by the ethanol within the system, even at a concentration incapable of producing a significant effect in water. This negative synergy is also seen in isoamyl alcohol, but in the opposite relationship with speed as seen with acetoin, here the friction increase is greatest at lower speed and least at higher speed. This again indicates the non-polar isoamyl alcohol is capable of disrupting some already present lubrication property but suggests it may do this to a different sort of interaction, or, it is capable of producing its own effect supplanting the relatively effective lubrication given by ethanol.

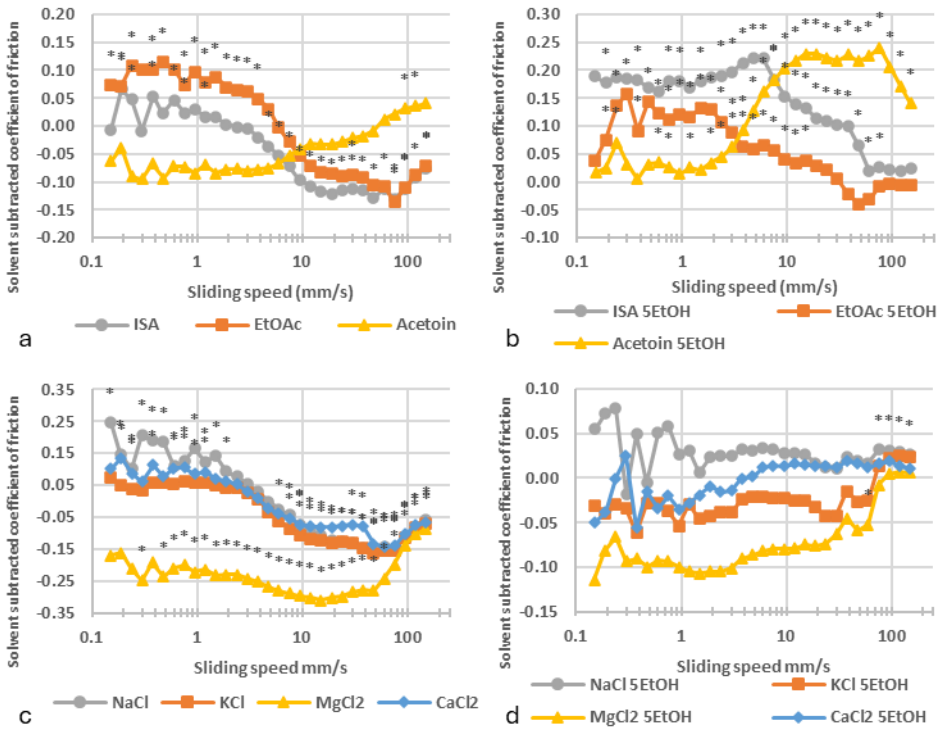

Figure 8. Solvent subtracted coefficient of friction over 0.1 mm/s to 150 mm/s with 1N axial downforce, for solutions of (A) Isoamyl alcohol 41 mg/L (ISA), ethyl acetate 32 mg/L (EtOAc) and acetoin 14 mg/L in water). (B) isoamyl alcohol 41 mg/L (ISA), ethyl acetate 32 mg/L (EtOAc) and acetoin 14 mg/L in 5% ethanol. (C) Sodium Chloride 250 mg/L, Potassium Chloride 1100 mg/L, Magnesium Chloride 265 mg/L and Calcium Chloride 140 mg/L in water. (D) Sodium Chloride 250 mg/L, Potassium Chloride 1100 mg/L, Magnesium Chloride 265 mg/L and Calcium Chloride 140 mg/L in 5% ethanol. * marks denote a statistically significant difference by one sided T test (p=<0.05) from the solvent alone.

Inorganic Systems

Inorganic salts have previously been shown to have positive lubrication properties (Garrec and Norton 2012) and are commonly manipulated in commercial beer products to produce desirable flavour profiles (Bellido-Milla et al. 2000; Hough et al. 1982). As such several chloride salts of group 1 and 2 metals were investigated, difference from solvent graphs were produced from generated Stribeck curves Figure 8C and Figure 8D. The first observation from this data is the significant increase in friction observed at many speeds from three of the salts tested, this is not expected, given previous data has shown significant reductions in friction from sodium chloride (Garrec and Norton 2012). Although the previous data was collected using a significantly higher concentration (5.8 g/L vs 0.23 g/L) and used a silicone elastomer and hydrophilic modified PDMS rather than the unmodified PDMS used here. These experimental changes may explain the differences observed in friction with the more hydrophobic unmodified PDMS used here presenting a less wettable surface for the aqueous ionic solutions to interact with, although sodium and magnesium chloride have been seen to interact with PDMS (Lipnizki et al. 2004) its interaction has not been quantified in terms of lubrication properties. Interestingly the differences observed once ethanol is included are much reduced compared to water systems, this suggests the salts are less able to disrupt already existing ethanol-dominated behaviour than the volatile organic molecules were seen to in Figure 7B. This limited disruption was not statistically significant under 59 mm/s for any data point while salts in water produced significant effects at all speeds for at least for the tested molecules. This indicates that the effect of ethanol lubrication may be stronger than the salts tested, and as such dominates the lubrication behaviour when present.

Organic Acid Systems

Organic acids, especially lactic acid have been seen to provide a major component of taste and texture in sour style beers (Dysvik et al. 2019) while lactic and acetic acid are the most commonly considered organic acids within beer, many others are also present (Klopper et al. 1986). As such a range of different organic acids were tested as lubricants, including acetic, lactic, citric, and succinic acids. These molecules cover a range of molecular mass while also representing a variety of chemical classifications within organic acids, with simple mono-valent acids such as acetic, simple hydroxy acids such as lactic, linear divalent acids in succinic and complex non-linear multi valent acids with citric acid. The generated solvent subtracted friction graphs are shown in Figure 9A and 9B. Initial observations with purely aqueous systems suggest that all the tested acids provide increased lubricity when compared to water alone, although the only showing consistent statistically significant reductions are seen at speeds above 5 mm/s. While the average observed reductions are greater at lower speeds, the error rates are higher, yielding non-significant differences. A more detailed analysis of the results shows that, at the highest speeds differences between molecules are limited, this was seen in most tests and is expected due to hydrodynamic forces present at these velocities. The results begin to spread out yielding some wide differences between them around 10 mm/s, here acetic and citric acid show similar behaviour, this is unexpected as they represent the smallest and the largest molecules with molecular weights of 60.05 and 210.14 respectively. This effect also cannot be explained by concentration, as citric acid is present are a 12.5-fold lower concentration by weight and 44 fold lower concentration by molarity. Given citric acid is a trivalent non-linear acid and acetic acid is a short chain linear mono-valent acid it would appear at first glance that the two share very little in terms of

properties. This difference is also clear in their chemical properties, with acetic acid exhibiting the highest logP at -0.17 and citric acid presenting the lowest at -1.64, additionally the pKas of 4.76 (acetic acid) and 3.13 (citric acid) represent the highest and lowest values for any tested acids. Overall, this suggests little in common between the two molecules, other than their tribological behaviour at specific speeds. The substances are seen to rapidly diverge once speeds of 7.5 mm/s or lower are tested, where citric acid is seen to behave more like succinic acid, which is structurally more similar and would be more likely the expected outcome. Similarly at lower speeds lactic acid and acetic acid show similar behaviour, again seeming to pair with its more structurally and chemically similar partner. Succinic acid meanwhile shows a similar overall shape to the results as the other acids, but with overall higher friction at most speeds.

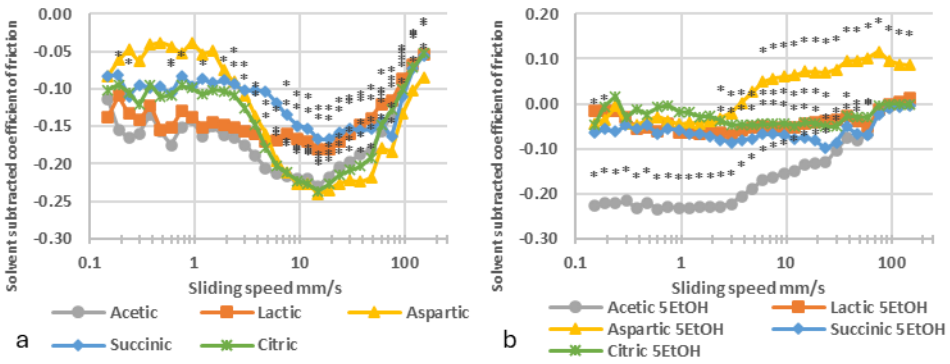

Figure 9. Solvent subtracted coefficient of friction over 0.1 mm/s to 150 mm/s with 1N axial downforce, for solutions of (A) Acetic Acid 2340 mg/L, Lactic Acid 1362 mg/L, Succinic Acid 166 mg/L and Citric Acid 186 mg/L dissolved in water), (B) Acetic Acid 2340 mg/L, Lactic Acid 1362 mg/L, Succinic Acid 166 mg/L and Citric Acid 186 mg/L in 5% ethanol. * marks denote a statistically significant difference by one sided T test (p=<0.05) from the solvent alone.

Upon addition of 5% ethanol tribological behaviour shows a marked change in all tested organic acids, Figure 9B shows the solvent subtracted coefficient of friction plots generated from these tests. From this it can be seen that the marked reduction in friction observed at speeds greater than 5 mm/s is significantly reduced in absolute value, while still showing statistical significance from the solvent alone. A major observation is the homogenisation of the behaviour seen with lactic, succinic, and citric acid, which in purely aqueous systems performed significantly differently compared to the much more uniform behaviour in the presence of ethanol. This trend does not continue with acetic acid, where the reduction in friction is even greater in ethanol containing systems than water only. This suggests some synergy is exhibited between the ethanol and acetic acid, which is not present in the other tested acids. A possible explanation is that acetic acid is miscible in ethanol, so the addition of organic modifier to the system would not reduce the solubility of the acetic acid, this is however discredited by the high solubility of all the tested acids in ethanol (Larrañaga et al. 2016). An alternative hypothesis would relate to the similar chemical structure of ethanol and acetic acid, differing only by the oxidation of the hydroxyl group to a carboxylic acid. This theory would rely on the similarity of the two molecules yielding a synergistic effect during tribo-corrosive layer formation, providing a film of similar molecules to coat the surfaces and reduce friction, the addition of acetic acid would allow for the filling of gaps not accessible to ethanol or water molecules. This similarity-based synergy hypothesis is supported by the behaviour of the other acids being similar, despite their significant structural and chemical differences, whereby only the acetic acid is capable of synergistically forming these films and while the others do not significantly disrupt the ethanol-based lubrication but also do not noticeably add to it.

Saccharide Systems

Traditionally saccharides, particularly dextrin content has been considered key in mouthfeel of beer (Langstaff and Lewis 1993) to investigate this, maltose, raffinose and medium chain maltodextrin (~7DE) samples were tested. The results of this analysis are shown in Figure 10A, here maltose and raffinose perform very similarly at all speeds, largely tracking the same behaviour with very few points being significantly different from water alone. The larger dextrin however, shows a marked reduction in friction between 7 and 95 mm/s sliding speed, this speed does cover most of the expected range of human tongue movements previously measured as 2.1-32.43 mm/s (Hiiemae and Palmer 2003), so it is within the range considered possibly relevant to *in vivo* perception. This reduction in friction is not sustained below 7 mm/s however, with the results rapidly returning to those observed for the smaller saccharides.

The addition of ethanol causes a significant shift in the observed friction, the previously significant reduction in friction at higher speeds with maltodextrin is lost and a non-significant reduction is observed at speeds below 75 mm/s with only occasional points being statistically distinct from 5% ethanol alone. This indicates that ethanol is able to alter the lubrication behaviour of the maltodextrin, but that it does not entirely dominate the behaviour, as some difference is still observed from water and 5% ethanol alone. Raffinose shows no statistically significant changes compared to the solvent, but a general slight reduction in friction is observed, suggesting ethanol is more able to replace the lubricity behaviour here. Maltose and ethanol exhibit a more unique behaviour, with small increases in friction over 0.2-37 mm/s with a number of significant differences seen around 1 mm/s.

This suggests that maltose, despite being at a lower concentration than raffinose is able to produce a unique lubricity behaviour which is detrimental to the overall friction behaviour.

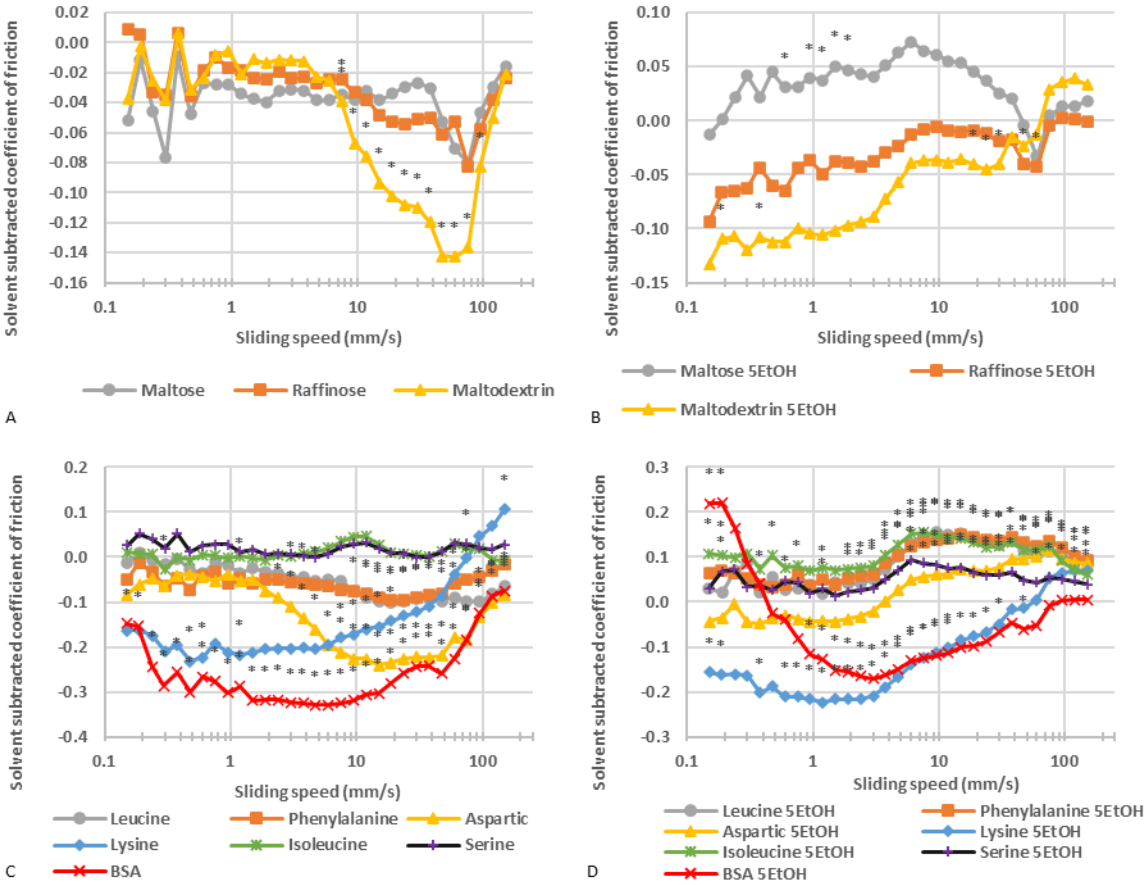

Figure 10. Solvent subtracted coefficient of friction over 0.1 mm/s to 150 mm/s with 1N axial downforce, for solutions of (A) Maltose 1270 mg/L, Raffinose 3140 mg/L, Maltodextrin 2480 mg/L dissolved in water, (B) Maltose 1270 mg/L, Raffinose 3140 mg/L, Maltodextrin 2480 mg/L in 5% ethanol), (C) Leucine 159 mg/L, Phenylalanine 99 mg/L, Aspartic Acid 82 mg/L, Lysine 80 mg/L, Isoleucine 159 mg/L, Serine 63 mg/L, Bovine Serum Albumin (BSA) 0.5% w/v dissolved in water. (D) Leucine 159 mg/L, Phenylalanine 99 mg/L, Aspartic Acid 82 mg/L, Lysine 80 mg/L, Isoleucine 159 mg/L, Serine 63 mg/L, Bovine Serum Albumin (BSA) 0.5% w/v dissolved in 5% ethanol. * marks denote a statistically significant difference by one sided T test (p=<0.05) from the solvent alone.

Amino Acid and Protein Systems

Amino acid content is not widely considered when discussing mouthfeel, but with the rise of low alcohol beers designed to provide nutritional benefits supplemented or naturally high amino acid and protein contents could be considered advantageous. As such, a range of amino acids were analysed as well as a model protein, bovine serum albumin (BSA). BSA was selected as it is an easily available model protein which has been extensively studied, with a moderate molecular mass of approximately 66,000 Da, it is also readily soluble in water without buffering with salts which were seen to alter tribological behaviour. Figure 2C shows the solvent subtracted coefficient of friction curves generated from, leucine, phenylalanine, aspartic acid, lysine, isoleucine, serine and BSA, at varied concentrations (see Table 1) all previously measured in a commercial beer (Fontana and Buiatti 2009). While an average protein content was taken from FDC Survey (U.S. Department of Agriculture 2022) and was consistent with previous studies (Abernathy et al. 2009) and was entirely made up with bovine serum albumin for analysis.

Initially it is noted that serine and isoleucine offer very limited changes compared to pure water, with no areas with statistically significant differences. These two amino acids similar behaviour is not predicted purely by their chemical properties, as serine is considered a polar but uncharged amino acid while isoleucine has a hydrophobic side chain, suggesting this property alone does not determine tribological behaviour. Following this leucine and phenylalanine also present with similar behaviour across the entire measured speed range, this would be more successfully predicted by the side chain properties, with both being classified as hydrophobic but both show noticeable variation to both serine and isoleucine,

again reinforcing that side chain polarity is not a primary determinant of tribological properties. Finally aspartic acid, lysine and bovine serum albumin exhibit unique properties, which do not closely match any of the others, aspartic acid is a fully charged amino acid and was also present at the lowest concentration, despite this, it shows a significant reduction in friction over a range of speeds primarily over 1.5 mm/s. At very low speeds, friction is still slightly reduced but not to a significant level. Lysine which is fully but oppositely charged to aspartic acid presents a somewhat inverse relationship with friction to aspartic acid, where at high speed friction is increased or slightly reduced, while at middle to low speed significant reductions are observed. This finding could suggest that fully charged amino acid side chains do have a more significant and potentially predictable impact on friction than uncharged side chains. Bovine serum albumin displays a fairly consistent reduction in friction at all speeds, although this reduction is greatest between 1 and 10 mm/s. The very large molecular mass of BSA means that in molar content 0.5% represents only 7.57 μM concentration, this is an unusually low concentration to yield such a marked reduction in friction and supportive of data on maltodextrin and sugars (Figure 10A/B) suggests molecular mass, especially when macromolecules are considered plays an important role in tribological effect. Given the large size of and heterogeneity within BSA it is likely areas of the molecules will be readily able to bind to some portion of many surfaces, potentially yielding a more surface agnostic lubricant than has been observed with smaller or more homogenous molecules.

This potential link between molecular of lubricating molecules and friction is supported by the data and correlations demonstrated here and lower molecular weight saccharides have previously been found not to meaningfully contribute to coating (Agorastos et al. 2023). The explanation of this phenomenon is likely linked to the physical distancing provided by one

molecule of these larger compounds. Where by a mono-film layer of bound protein or large saccharide physically distances the surfaces by a greater distance than a smaller molecule is capable of, purely as the intra molecular bonding is strong enough to resist friction forces, instead failing at the molecules connection to the surface. This greater physical distance reduces the chances and severity of asperity contact, thus reducing friction (Hsu 2004; Willermet et al. 1995). To more completely examine this a wider range of molecular sizes, utilized at equivalent molar concentrations could be attempted, this may also identify a critical size point, where by the physical distancing becomes largely irrelevant due to the comparative size of the surface imperfections.

Ethanol is commonly used in biology to precipitate proteins and is also somewhat effective at precipitating amino acids (Yoshikawa et al. 2012) due to the very poor solubility of these molecules in organic solvent and their ability to unfold the complex tertiary structures of proteins. As such ethanol could be predicted to not have positive effects on the tribology of proteins and amino acids, this was largely found to be correct, with isoleucine, leucine, phenylalanine and leucine showing largely similar patterns in lubricity to each other and all showing higher friction at all speeds than just 5% ethanol. The ethanol's lubrication properties appear to be disrupted by the presence of the amino acids, which are unable to fully overcome the ethanol's effect to demonstrate their own intrinsic lubrication properties while also preventing the ethanol from fully exhibiting its own. This yields a statistically significant and higher coefficient of friction at most test speeds while serine, demonstrates higher, but not significantly higher friction. Similarly to in water systems, aspartic acid, lysine and BSA display unique behaviour, while again all being distinct from each other, aspartic acid shows moderate increases in friction at speeds above 37 mm/s but eventually comes

down to yield a slight reduction at speeds less than 3 mm/s. This could suggest the fully charged and negative acid is able to form relatively stable attractions to positively charged elements in the metallic surface, which do not degrade under high friction stress, providing a successful tribo-corrosive effect. Also of interest is lysine, which as opposed to results obtained in fully aqueous systems demonstrates a similar profile shape to aspartic acid, but with a Y axis offset with significantly reduced friction. Here it is possible the ethanol has changed the selectivity of the binding of lysine, causing it to better form tribofilms which again are highly stable in high friction environments. Bovine serum albumin seems to demonstrate a different effect, here a similar profile to lysine is observed until 4.7 mms/ where the samples significantly diverge, with observed friction in BSA samples rising rapidly to eventually be the highest measured friction after 0.23 mm/s, this rapidly increasing friction value suggests the previously stable tribo-films of protein are fracturing, yielding a roughened surface of partially bound molecules which are repellent to the ethanol due to their charged nature. This repulsion prevents the ethanol from simply becoming the major lubricant, instead producing a mixed system or partially bound protein films with ethanol filling gaps where binding is possible.

Correlation analysis

To further investigate the potential basis for the observed single component system effects a range of chemical properties were plotted against coefficient of friction at three selected values, 95, 12 and 1.2 mm/s. These values were selected to cover a wide range of values and also represent the main observed regions of the Stribeck curves generated, these being hydrodynamic, mixed and boundary (Figure 11). The values also fully cover the previously

quantified average range of movements within human mouths (Hiiemae and Palmer 2003) as well as representing a higher value (95 mm/s) that while on the higher end of previously measured values is still possible *in vivo*. The chosen chemical properties were, molecular mass, topographic polar surface area (TPSA), logP and pKa, molecules which did not possess a specific property were not included in that analysis, for example logP for inorganic molecules.

Initially all molecules were correlated at once, from this no significant correlations were observed, likely due to the wide range of chemical properties seen when comparing across such varied molecules. It was also noted that the very large size of bovine serum albumin significantly biased the molecular mass based plots. The strongest correlations were observed with pKa at 12 mm/s, with a correlation of R^2= 0.2465 and pKa at 95 mm/s with an R^2 of 0.2266, although similarly to molecular mass this was severely biased by the pKa of ethanol. The trend does overall suggest molecules with a higher pKa offer better lubrication properties than more strongly acid ones but the correlation is not strong.

In contrast to pKa, logP was found overall to have very little correlation with friction, producing R^2 values of 0.071 at 95 mm/s, 0.021 at 12 mm/s and 0.00005 at 1.2 mm/s, this indicates that when taken as a whole logP is not able to predict lubrication behaviour at all. The second strongest correlation was found with molecular mass at 1.2 mm/s, where and an R^2 of 0.2066 was obtained, indicating a weak negative overall correlation between molecular mass and friction, although the correlation was significantly less strong at 95 mm/s, indicting molecular mass is significantly more relevant at slower speeds.

Further to this general analysis each class of molecule was separated and plotted in the same manner, this yielded more focused plots of far more chemically similar molecules. From this the lubrication capability of the saccharide was found to be directly correlated with the molecular mass Figure 3 shows the results of coefficient of friction plotted against molecular mass, using co-efficient of friction from three decades of measurement, 95 mm/s, 12 mm/s and 1.2 mm/s. At 95 and 12 mm/s the correlation is stronger and negative (R^2 of 0.8154 and 0.996 respectively), molecular mass is a key factor in lubrication for saccharides. The degree of reduction in friction is significantly greater at 12 mm/s than 95, suggesting that at higher speeds the analyte has less effect most likely due to the domination of behaviour by hydrodynamic factors.

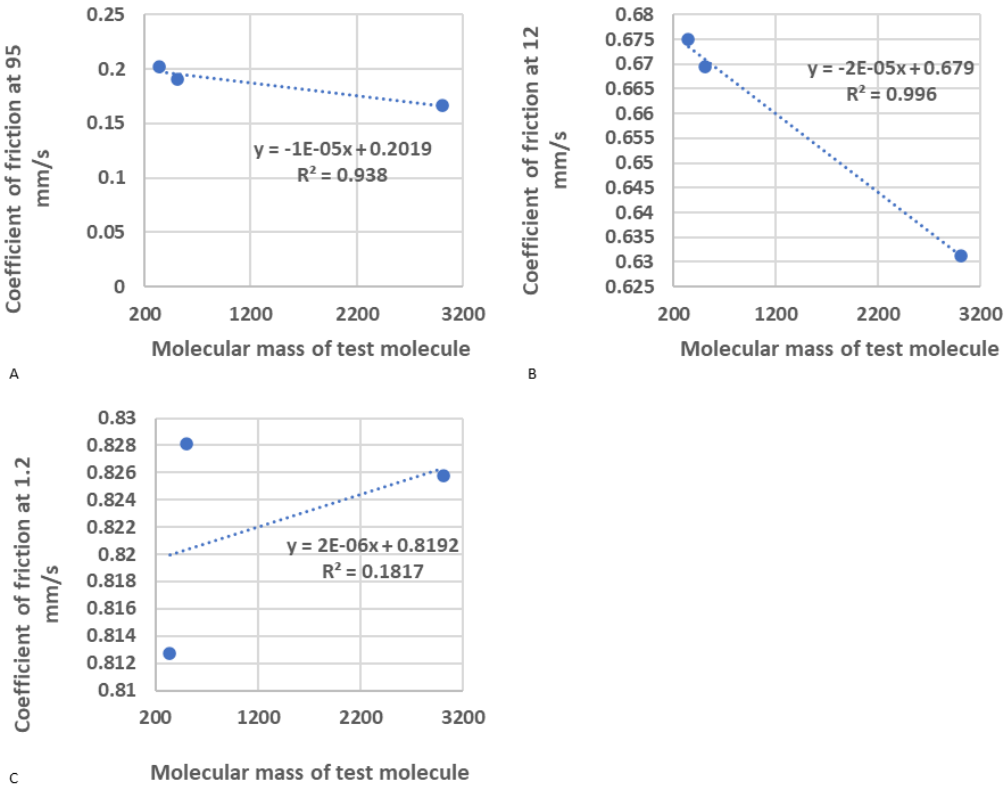

Figure 11. Correlation analysis of coefficient of friction at selected sliding speeds vs the molecular mass of the tested saccharide molecule. Molecular weight of maltodextrin is an estimated based on 6.5 DE average and the formula (180*n-18*(n-1)) where n is degree of polymerisation. (A) Molecular mass plotted against coefficient of friction at 95 mm/s sliding speed. (B) Molecular mass plotted against coefficient of friction at 12 mm/s sliding speed. (C) Molecular mass plotted against coefficient of friction at 1.2 mm/s sliding speed.

When only volatile organic molecules were compared several significant correlations are observed, the strongest of which was with TSPA at 95 mm/s, this yielded a positive correlation with R^2 of 0.8154, molecular mass also showed a moderate correlation (0.4829) but at 12 and 1.2 mm/s rather than 95 mm/s. This difference between TSPA and molecular mass is interesting, suggesting that increased polar surface area is important at both 95 and 12 mm/s but becomes irrelevant at very low speeds while molecular mass remains relevant.

Similarly to volatile organic molecules, organic acids showed some correlations with molecular mass and TSPA while showing very poor correlations with logP and pKa, this was more unexpected within organic acids, as pKa is a more relevant statistic to these more readily de-protonatable compounds. Given the multivalent nature of several of the acids tested, the weakest pKa for each molecule was also considered as opposed to just pKa1, this interestingly did yield significant correlations at all speeds, with 0.5993 at 95 mm/s, 0.1189 at 12 mm/s and 0.5656 at 1.2 mm/s. Beyond the R^2 value it is observed that a higher pKa results in lower friction at 95 mm/s, while a lower pKa results in reduced friction at 1.2 mm/s, this supports an idea that non-polar interactions derived from still protonated acid groups are important at high speeds, while at lower speeds polar interactions become more important to lubricity stemming from deprotonated acid groups. This link could be a biproduct of the higher friction forces at low speeds, meaning non-polar interactions aren't able to remain in place, causing film deformation or damage, resulting in less optimal boundary lubrication.

With the amino acid class being structurally and chemically the most varied it is interesting that some correlations are observed within this group that were not seen in more uniform classes. For example, LogP yields a weak correlation with friction at 12 mm/s (0.2471) which is significantly stronger than was seen for any other group of molecules using this property, although given the poor correlations observed for other groups, it is difficult to draw conclusions from this finding. Also of note for amino acids, pKa was the least relevant predictor of tribological behaviour, with no correlations at any speed being greater than 0.1, this suggests protonation is not a major factor in the test system. Again as seen with the volatile organic molecules TSPA shows some correlations, which are stronger than those seen

with purely molecular mass, suggesting that polarity is important but when combined with the pKa data, seemingly polarity rather than fully charged interactions are important here.

Finally the tested inorganic chloride salts, with the fewest comparable properties available, molecular mass yielded a single weak correlation (R^2 0.1157) and only at 1.2 mm/s. Formal charge of the cation did however yield some higher correlation values, suggesting a weak connection between charge and friction, but given only mono and divalent ions were tested data is limited with which to draw significant conclusions.

A summary of the observed correlations is presented in table 3. This table includes the nature (positive or negative) of the correlations as well as the R^2 values obtained when the chemical property was plotted vs coefficient of friction at the previously mentioned sliding speeds.

Property	R^2 95 mm/s	R^2 12 mm/s	R^2 1.2 mm/s
Molecular mass (all)	0.0322	0.1602	0.2066
Molecular mass (saccharides)	0.9380	0.9960	0.1817
Molecular mass (acids)	0.2196	0.0959	0.4290
Molecular mass (inorganic)	0.0153	0.0462	0.1157
Molecular mass (VOC)	0.1087	0.4829	0.4141
Molecular mass (amino acids)	0.0141	0.1806	0.2194
TSPA (all)	0.0019	0.0123	0.0125
TSPA (saccharides)	N/A	N/A	N/A
TSPA (acids)	0.2183	0.0894	0.4322
TSPA (inorganic)	N/A	N/A	N/A
TSPA (VOC)	0.8154	0.4235	0.0000
TSPA (amino acids)	0.0131	0.3799	0.1583
logP (all)	0.0711	0.0211	0.0000
logP (saccharides)	N/A	N/A	N/A
logP (acids)	0.0578	0.1040	0.2406
logP (inorganic)	N/A	N/A	N/A
logP (VOC)	0.1254	0.0647	0.5215
logP (amino acids)	0.0045	0.2471	0.0840
pKa (all)	0.2266	0.2465	0.1952
pKa (saccharides)	N/A	N/A	N/A
pKa (acids)	0.0325	0.0420	0.1980
pKa (inorganic)	N/A	N/A	N/A
pKa (VOC)	N/A	N/A	N/A
pKa (amino acids)	0.0591	0.0805	0.0012

Table 3. Summary of correlation analysis between various properties and coefficient of friction at 95, 12 and 1.2 mm/s. R^2 values are given to four decimal places, red text indicates a correlation is negative (a greater value for the property yields lower friction), while green text indicates positive correlation (a greater value for the property yields higher friction).

Double molecule systems

Using the data obtained from single molecules combinations were selected to investigate potential synergy or anti-synergy effects based on chemical properties and tribological behaviour, again these mixtures were run in purely water based systems as well as with 5% ethanol to investigate its role. To this end 44 different combinations were tested each with and without ethanol and could largely be categorized into additive mixtures, where the two molecules tribological effect was purely additional to each other, synergistic but not directly additive, anti-synergistic where the result exhibits more friction than either molecule alone and unique, where an interaction that yields a different shaped curve was observed. To visualise this, Stribeck curves were produced showing the measured coefficient of friction compared to the average of the two constituent parts alone. For example Figure 12a shows a plot of a sodium chloride and ethyl acetate mixture with and without ethanol, also plotted is the predicted outcome if the individual friction results were simply averaged together. From this figure it can be seen that a slight difference in friction is observed from the predicted values at speeds below 2 mm/s, significance markers indicate that the difference between the average of individual components and the mixture is statistically significant in 1 tailed T-test ($p=<0.05$). Once ethanol is included in the system there are no significant differences between the predicted values and the measured values for sodium chloride and ethyl acetate, this is an example of net negligible effect molecules. Alternatively, Figure 12b demonstrates a negative synergy interaction, where in friction is significantly higher than predicted at all speeds under 100 mm/s, this relationship is exhibited where lactic and acetic acids are mixed, individually both proved effective at reducing friction, but when added together this effect is significantly negated. In this instance it is likely due to the stronger

acetic acid forcibly protonating the weaker lactic acid, which is then unable to form its own effective tribofilms based on polar interactions and also negatively impacts the acetic acid's capability to do the same by forming non-polar based interactions with the surfaces. It is also noted that the mixture has increased friction compared to either single acid alone in water. With the addition of ethanol a similar effect is seen, where the highly effective lubricity observed with ethanol acetic acid mixtures is reduced significantly at many speeds by the interactions with lactic acid, showing a difference between the predicted and measured values, which is statistically significant above 1.8 mm/s. This is hypothesised to be caused by the now non-polar lactic acid disrupting the ethanol acetic acid film formation by forming its own non-polar interactions making the polar ones seen in ethanol and deprotonated acetic acid less viable.

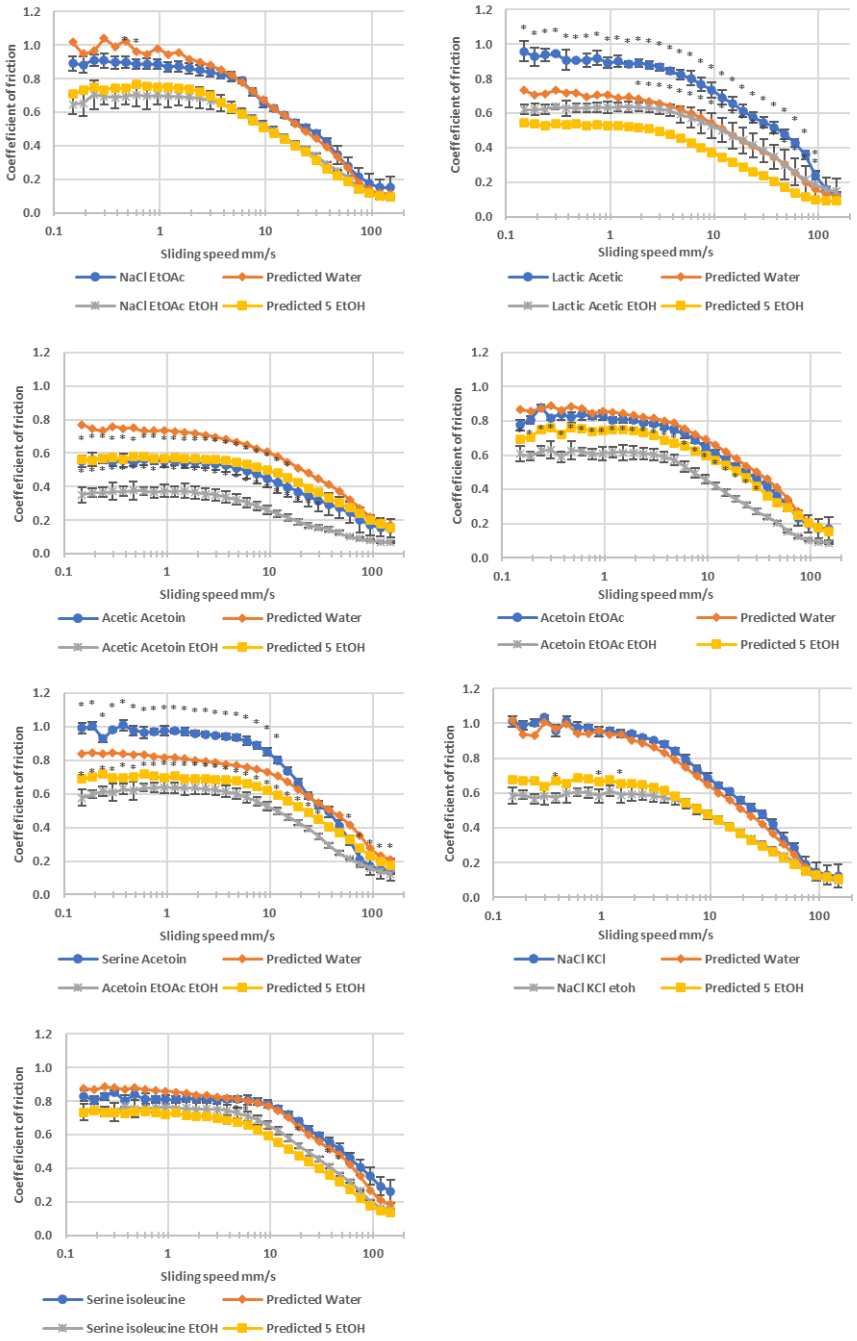

Figure 12. Stribeck curves generated using 1N axial force on mixed systems of molecules, along with the predicted values for that mixture, which were estimated by simply averaging the coefficient of friction values for both substances separately. Error bars represent 1 standard deviation, * marks indicate a statistically significant difference ($p<0.05$) at that data point compared to its predicted value. 4A sodium chloride 230 mg/L + 32 mg/L ethyl acetate. 4B lactic acid 1362 mg/L + acetic acid 2340 mg/L. 4C acetic acid 2340 mg/L + acetoin 14 mg/L. 4D Acetoin 14 mg/L + ethyl acetate 32 mg/L. 4E Serine 63 mg/L + acetoin 14 mg/L. 4F Sodium chloride 230 mg/L + potassium chloride 1100 mg/L. 4G serine 63 mg/L + isoleucine 159 mg/L.

In contrast to its mixture with lactic acid, when mixed with acetoin, acetic acid demonstrates a synergistic effect both with and without ethanol, this is despite the low concentration of acetoin present, Figure 12C shows the observed friction behaviour for this mixture. Here there is a less substantial effect at medium to low speeds whereby the measured value does not correspond to the prediction from averaging the molecules lone tribological behaviour. The results above what is predicted, especially with ethanol, was found to be unusual and gives a clear demonstration of the acetic acid, acetoin and ethanol forming a more effective lubrication system than is capable of being formed with any two of the components. The differences from predicted values were not found to be significant at very high speeds, where previous analysis indicated a link with less polar molecules providing positive lubrication, this lack of significance is likely due to hydrodynamic factors combined with the low concentration of acetoin used along with the high content of polar ethanol and acetic acid. The synergy at lower speeds is however harder to explain, one hypothesis is the acetoin is able to fill the less polar binding areas where ethanol and acetic acid are less able to form tribofilms, likely centred around the PDMS surface rather than the stainless steel. To further investigate this acetoin-based effect, acetoin and ethyl acetate mixtures were examined, thus providing a second non-polar molecule to assist in dissecting the interactions. With the addition of ethyl acetate no significant deviation was observed from the expected values in water based systems Figure 12D, this is largely expected, as neither ethyl acetate or acetoin showed any significant changes to friction values in water. With ethanol containing systems both acetoin and ethyl acetate alone were seen to disrupt the ethanol-based lubricity, causing increased friction when compared to ethanol and water alone. This synergy now between polar ethanol and less polar ethyl acetate and acetoin goes to indicate further than

a heterogeneity of lubrication molecules can produce a positive effect on lubricity even when additives are at low concentrations.

Due to the interesting synergy displayed acetoin was also tested with serine, this is a chemically more complex mixture, given the zwitterionic nature of serine along with its hydroxyl group. Serine alone is not seen to cause significant changes in lubricity in pure water systems (Figure 10C), but it does show some slight increases in friction at medium speeds with ethanol (Figure 10D). When combined with acetoin a significant anti-synergy is observed in water (Figure 12E), showing an increase in friction when compared to the predicted values, this was not observed in the previous mixtures with acetoin. The mechanical basis for this change is not clear as neither serine or acetoin demonstrated a similar interaction with any other tested compound, although the specificity of this interaction would suggest that there is some unique molecular interaction generated between the two that has a negative effect of lubrication. In contrast to the increased friction measured in water systems once ethanol is considered serine and acetoin show a significant reduction in friction over the predicted value (Figure 12E), this again supports the hypothesis that the heterogeneity of the system allows for more effective lubrication with a range of molecular sizes and properties available to form functional tribofilms. This theory seems to primarily be applicable to systems containing ethanol, with several other mixtures demonstrating limited effect in purely water-based systems while exhibiting a significant reduction in friction when ethanol is considered. More unexpectedly some synergy is also seen between sodium and potassium (Figure 8F) with ethanol. This is less predictable due to both molecules being inorganic and very similar in properties, the ability of the mixture to

lubricate beyond averaging the individual outcomes again suggests that particularly with ethanol, heterogeneity even to a limited level can be beneficial for lubricity.

There are however several molecular mixtures showing anti-synergy with ethanol which lack the chemical explanation presented with lactic and acetic acids. The amino acid isoleucine produces anti-synergy with both serine (Figure 12G) and lysine (Figure 12H), although mostly not statistically significant in nature, the trend especially with lysine is to produce higher friction with ethanol and even in pure water systems. Chemically this is more difficult to explain than previous examples, with both molecules being amino acids, but from different classifications, lysine is a fully charged basic amino acid, while isoleucine has a hydrophobic side chain. This could be expected to allow the non-polar plus polar interactions previously seen to have positive effects on friction in acetoin mixtures, but in these examples it does not. The relatively high polarity of isoleucine could represent the cause of this difference, as despite being considered non-polar for an amino acid, it is still a highly polar molecule (when compared to acetoin) and steric hinderance of the non-polar side chain between carboxyl and amine termini is likely to play a role.

The effect of serine isoleucine also proves challenging to explain with the previous hypothesis, as both serine and isoleucine are considered non-polar amino acids, although comparatively are both relatively polar with logP of -3.07 and -1.7 respectively. This makes them in fact overall significantly more polar compared to truly low polarity molecules also tested, their ability to form acid and base related interactions also makes their binding chemistry more complicated in mixtures with possible binding to both negative and positive surfaces and potentially with the non-polar side chain amino acids to non-polar surfaces too.

While this binding ability may appear to be advantageous in forming stable tribofilms this seems not to be the observed case.

While film thickness has not been measured for the specific systems tested here, it has previously been demonstrated that thickness is variable based on composition and the conditions used for formation (Dawczyk et al. 2019). Thickness is not however, the sole determinant of lubrication quality or even stability of the film (Morina and Neville 2007), also of importance is the strength of attachment present, which is largely dependent on the chemical composition of the surfaces and lubricant (Summers et al. 2020) and the conditions under which they form (Willermet et al. 1995). The thickness of films combined with the difficulty in their erosion becomes determinant of lubrication quality in pure boundary lubrication, largely being linked to their continued integrity and thus smoothing effect (Zhmud et al. 2014). Relating this to the results for amino acids, further reinforces the unexpected nature of the results, as electrostatic based interactions have been seen to be form stronger tribofilms than simple Van Der Waals forces (Ismail and Bagheri 2017) and such polar based interactions would be expected from the zwitterionic amino acid molecules. The relatively weak nature of the acid/amine groups within the amino acids may explain the relatively weak lubricity as generally stronger acid or hydroxyl are considered relevant to binding stainless steel (Simic and Kalin 2013). It is possible that these amino acids are in fact using their lower polarity side chains to form non-specific interactions to PDMS surfaces, rather than the stronger polarity-based ones with metal surfaces, but this requires further investigation to determine.

Macromolecule Systems

The macromolecules tested, maltodextrin and bovine serum albumin both displayed unique behaviour, with Stribeck curves showing markedly different forms to the smaller molecules. BSA is seen to have an unusual profile in Stribeck curves produced using it, the generally expected sigmoid shape observed with all other tested samples (Figure 13A). This shape was also distinct from those seen in maltodextrin samples (except when they also included BSA), indicating that the profile is unique either to proteins, or unique to molecules with especially high molecular mass. The unique appearance was preserved in most mixtures attempted with BSA, changes being limited to the shift in Y axis position rather than causing significant changes to the overall shape, this suggests that the lubrication mechanism for molecules this size is very robust and resists disruption by other potentially interfering molecules, to a much greater extent than ethanol is capable of, despite being present at a far lower concentration.

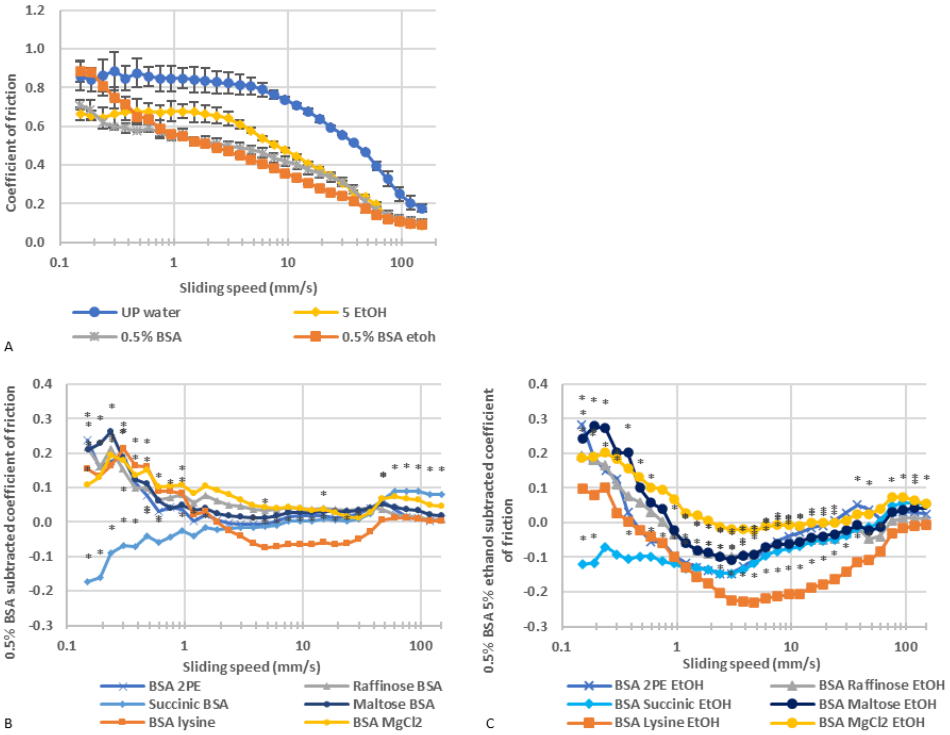

Figure 13 (A). Stribeck curves generated for water, 5% ethanol, 0.5% BSA in water and 0.5% BSA in 5% ethanol over 0.1 mm/s to 150 mm/s with 1N axial downforce. Error bars represent 1 standard deviation.

(B) Solvent subtracted curves generated from mixtures of BSA with; 2-phenylethanol, raffinose, succinic acid, maltose, lysine and magnesium chloride in water. * marks denote a statistically significant difference by one sided T test (p=<0.05) from 0.5% BSA in water.

(C) Solvent subtracted curves generated from mixtures of BSA with; 2-phenylethanol, raffinose, succinic acid, maltose, lysine and magnesium chloride in 5% ethanol. * marks denote a statistically significant difference by one sided T test (p=<0.05) from 0.5% BSA in 5% ethanol.

Ethanol also interacts with BSA in a unique way, only causing significant changes to lubricity at very slow sliding speeds, here ethanol appears to be disruptive to BSA based lubrication, causing a rapid increase in friction below 0.75 mm/s causing an upward flick to the end of the curve shape which is not present in BSA/water systems. The unique shape of Stribeck curves generated using BSA suggests its lubrication mechanism is significantly different to

that of the other molecules tested, to investigate this a range of small molecules were tested in combination with BSA with and without ethanol Figure 13B and 13C. The immediate observation from these tests is that significant effects are primarily located at the very low test speeds, as well as speeds above 47 mm/s, the middle range of speeds shows few significant changes with any of the test molecules, with the exception of lysine. Lysine when tested alone was seen to produce a significant reduction in friction in both water and 5% ethanol and seems able to provide that enhancement in the BSA water systems. This capability also partially matches that seen for succinic acid, which is also seen to reduce friction alone, however in succinic acids case it has no significant effect at the speeds lysine did, instead showing a very large reduction in friction at very low speeds. The contrast with these two molecules suggests that the charge of the cooperating molecule has a significant effect, lysine is a basic amino acid so is positively charged from its second amine group accepting H+ ions, succinic acid is a divalent carboxylic acid, so in these experiments would be negatively charged from donating H+ ions. All the other test molecules except magnesium are uncharged and all follow a more similar pattern. This data suggests that formal charge of organic molecules could be a factor in some elements of friction behaviour when heterogeneous macromolecules are present. The increase in friction observed at low speeds with all molecules other than succinic acid is however still of interest, as the majority of these molecules had limited to no significant effect when tested alone, but with BSA are able to cause as significant increase in friction but only at very low speeds. This specificity suggests they are weakening tribofilms which at high friction are more likely to fracture while the negatively charged succinic acid is able to replace failing BSA films producing a novel lubrication behaviour, or provides an increase in stability above BSA alone, meaning films perform better at higher friction force than in tests without a second molecule type. The

clustering observed between the molecules causing increased friction, irrespective of chemical class or other properties strongly suggests that in this case the common behaviour is disruptive, while succinic acid provides an outlying property.

Upon addition of ethanol to the systems interactions are altered, here several more molecules show a significant reduction in friction are middle speeds, with maltose, raffinose, succinic acid, 2-phenylethanol and lysine all having significant reductions. Only magnesium chloride is not seen to change friction between 1.8 and 30 mm/s, this is potentially explained by its inorganic nature, making interactions with ethanol less prominent than with the organic molecules tested. Although magnesium is still able to disrupt lubrication at high friction forces seen at lower speeds, salt concentration is important in protein folding and tertiary structure, so a possible explanation for this observation is its ability to alter the protein folding, producing a less stable tribofilm which is more readily damaged by stress, thus increasing friction forces. Succinic acid and lysine still demonstrate outlying behaviours in the ethanol system, with succinic acid showing a similar but less pronounced profile compared to non-alcohol containing tests. Here a significant reduction in friction is observed in the very low speed region, but it is less frequently significant and represents a lower absolute reduction than was observed without ethanol present. Lysine however demonstrates the opposite behaviour, when ethanol is present the friction reduction is significant for a greater range of speeds, as well as being greater in absolute reduction, indicating that basic interactions are enhanced by the presence of ethanol, potentially relating to the folding status of the protein being altered by the environmental pH increasing allowing for effective lubrication at low to high speeds, but again causing increased friction at very low speeds, although in this case not significantly. From this ethanol is seen to enhance

cooperation with lysine while reducing but not removing cooperation with succinic acid. The mid speed friction reduction observed in all organic samples also suggests that this speed is the most susceptible to change, this is of note due to this region being the most in vivo relevant of the range tested.

Following the unusual results obtained with BSA maltodextrin mixtures were tested Figure 14B, the far more homogenous nature of maltodextrin as a polymer being made only of glucose subunits with α 1-4 glycosidic bonds this offers less diversity of possible binding mechanisms than would be seen in a heterogeneous polymer such as BSA. The maltodextrin utilized here has a calculated molecular mass of 3008 Da, making the polymer significantly smaller than the 66,000 Da estimate for BSA. Interestingly the inorganic salt behaves in a different way than was observed with magnesium in BSA tests, here the chloride salt shows some small but significant increases in friction at 75 mm/s and higher speeds, but then only shows minor and non-significant increases over the rest of the tested speed range. This difference from behaviour seen with BSA mixtures could be linked to the lower degree of tertiary structure in maltodextrin compared to the much larger and more complex BSA, whereby the inorganic ions are less able to alter tertiary structure and so fail to disrupt tribofilm stability and do not provide very significant properties of their own. Similarly to sodium, isoamyl alcohol causes very limited changes in friction with only some significant changes between 94 and 120 mm/s although in contrast with sodium these changes are to reduce friction, not increase it. Iso-amyl alcohol represents a significantly non-polar molecule and appears to be unable to cause impactful interactions when maltodextrin is present, this is possibly due to polar maltodextrin polymers already coating the surfaces and due to the much higher concentration they provide the dominant behaviour of the system.

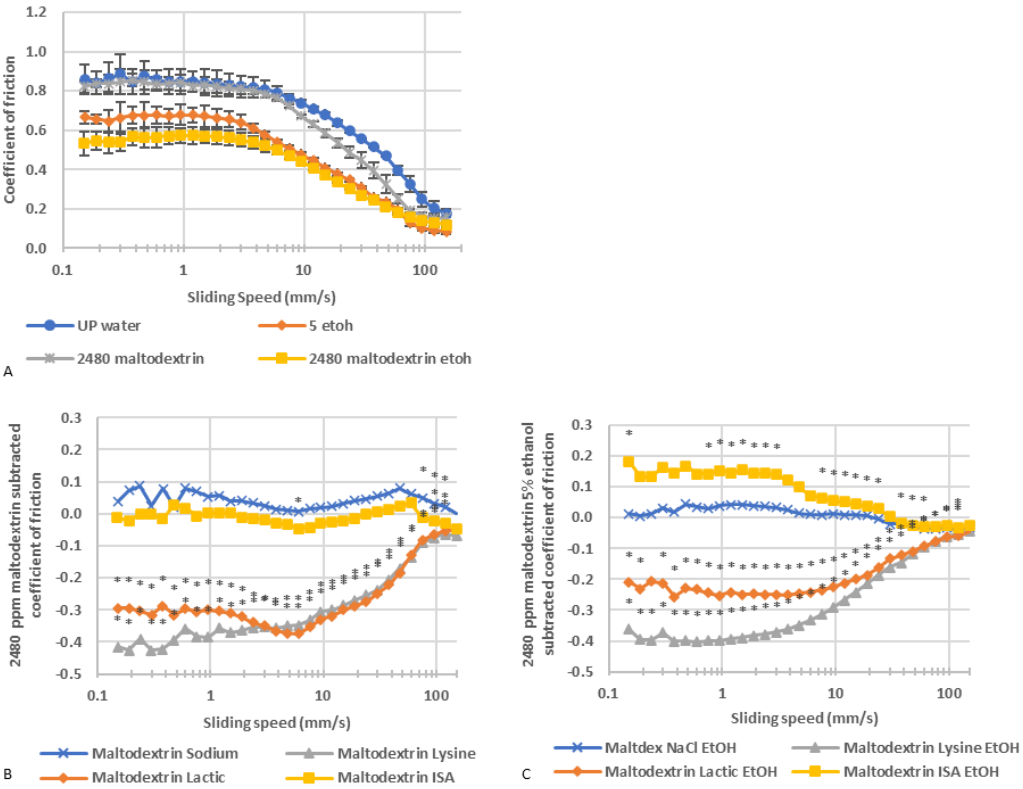

Figure 14 (A). Stribeck curves generated for water, 5% ethanol, 2480 mg/L maltodextrin in water and 2480 mg/L maltodextrin in 5% ethanol over 0.1 mm/s to 150 mm/s with 1N axial downforce. Error bars represent 1 standard deviation. (B) Solvent subtracted curves generated from mixtures of maltodextrin with; sodium chloride, lysine, lactic acid and isoamyl alcohol in water. * marks denote a statistically significant difference by one sided T test (p=<0.05) from maltodextrin in water.

(C) Solvent subtracted curves generated from mixtures of maltodextrin with; sodium chloride, lysine, lactic acid and isoamyl alcohol in 5% ethanol. * marks denote a statistically significant difference by one sided T test (p=<0.05) from maltodextrin in 5% ethanol.

In contrast to isoamyl alcohol and sodium chloride, lactic acid and lysine mixtures with maltodextrin show significant friction reductions over all tested speeds indicating a noticeably more effective lubrication profile. A somewhat similar effect was observed with lysine and BSA, where a significant reduction was measured between 29 and 3.8 mm/s, the far greater range of significance as well as the larger absolute value of reduction suggests

lysine is more effective as a lubricity partner with maltodextrin than with BSA. This result is interesting, as BSA itself contains lysine (~12% by mass) (Spahr and Edsall 1964) so it could be expected that the similarity between BSA and lysine would induce a more effective interaction. Similarly succinic acid was examined with BSA and produced a significant reduction in friction, but only at high friction forces seen at low speed, while lactic acid is able to produce a stronger absolute reduction in friction as well show significance over a greater range of speeds. This demonstrates the complexity of predicting tribological behaviour is only increased when macromolecules are to be considered, with behaviours differing significantly based on the exact molecule in question.

To further examine the interactions discussed here a schematic diagram of proposed molecular alignments was constructed (Figure 15). This figure approximates the likely molecular alignment of various possible interactions when forming a monolayer film, for example many single molecule systems are likely to form type A interactions, whereby the molecules are able to alignment in such a way that they form a relatively cohesive film (subject to sufficient concentration). It is however likely that highly polar molecules, especially those which are polar in multiple places could face some polarity based hinderance to their tessellation, the strength of this effect would be based upon the level of polarity/charge and also the physical shape and size of the molecule, if sufficiently appart some tessellation is predictable. Amino acids however would be predicted to form type C interactions, this being due to their zwitterionic nature, allowing for the positive termini to align with negative termini of other examples of the same molecule, representing inter molecular heterogeneity. Alternatively to inter molecular heterogeneity, some benefit may be gained from intra molecular differences, (D) shows the predicted interactions between

two different compounds with alternate charges/polarities. This polar synergy was not observed in any of the tested mixtures, likely as positively charges molecules are uncommon in beer due to its pH, the addition of organic bases or molecules with positively charged elements would likely enable this interaction type. Type E interactions predict purely steric concerns for mono-film formation, this is possible even when molecules are compatible based on charge or polarity, but physically form energetically unfavourable states with poor attraction to each other, allowing for film deformation more easily. These type E interactions were likely seen in acetoin mixtures, as the molecule contains both polar and non-polar regions but frequently displayed negative synergy, suggesting its physical shape could be a cause. Contrary to (E) it is also possible that addition of a differently shaped molecule could assist coverage/tessellation, here two molecules are able to form a specific confirmation where film strength increases compared to one of them alone. Although in the example shown in Figure 15, one of the molecules would form a stable film with few gaps, the other is not capable of this, in this instance it may be seen that the lubricity of the square shaped molecule would be negatively affected by addition of the cut away circle, but when compared to just the circular molecule an improvement would be observed.

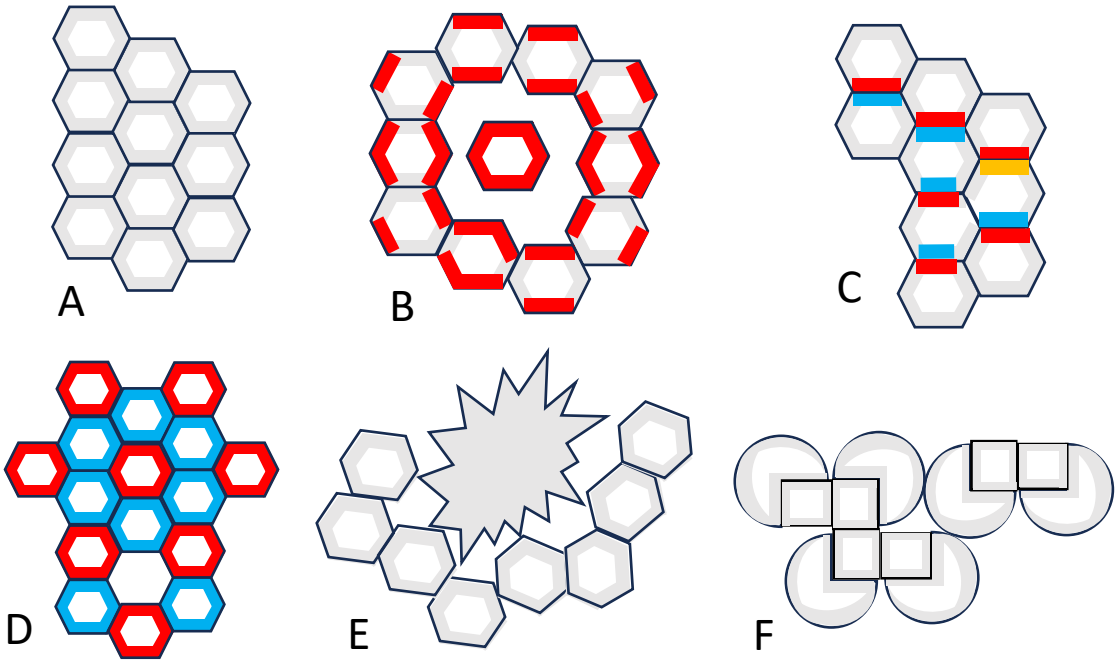

Figure 15. Schematic representations of predicted interactions between compounds forming tribofilms based on observations from this work and general electrostatic interaction chemistry. (A) showing non-polar interactions with good molecular tessellation, (B) alignment with matching polar compounds disrupting film formation and strength, (C) potential synergy from oppositely polar regions of the same molecules forming interactions together, (D) synergy from opposite polar regions of different molecules, (E) anti-synergistic effect of physical steric hinderance on film formation with heterogeneous molecules and (F) potential synergistic effect of heterogeneously shaped molecules. Negligibly charged regions are represented in grey, negative regions are represented in red, while positive regions are represented in blue.

Conclusions

The analysis conducted in this study demonstrates a previously undescribed potential role for volatile organic aromatics in tribology and mouthfeel at concentrations previously measured in commercial beer products. This data also presents findings around ethanol's contribution to lubricity and demonstrates mixtures in which it would be predicted to have negative effects on mouthfeel rather than the generally accepted positive effects, indicating that in low and no alcohol beer the loss of ethanol alone is unlikely to be the sole cause of and changes in observed mouthfeel. It was also found that inorganic salts effects on lubricity are unpredictable, even within group 1 and 2 chlorides, despite their similar chemical properties. As may be expected, lactic acid was found to have a significant effect on lubricity, but so were less abundant acids, such as citric and succinic which are often not considered when discussing mouthfeel. It was also noted that a single di or trisaccharide sugar at beer relevant concentrations was unable to significantly alter tribological behaviour while showing unexpected interactions with ethanol. In contrast maltodextrin was seen to provide significant reductions in friction, suggesting polysaccharides may play a larger role than residual sugar in standard beer products. Proteins and amino acids were also seen to have interesting effects on tribological behaviour, suggesting their manipulation could be beneficial to product optimisation, although this requires more detailed analysis in more complex systems.

It was also demonstrated that predicting tribological behaviour is challenging, even with very simple systems, interactions are hard to predict even when both molecules effects have been independently quantified. This unpredictability was exacerbated when macromolecules were

considered showing that predictive tribology even in simple systems is still a significant challenge for future work.

Funding Statement

This work was funded by the Adrian Brown Scholarship in brewing from the University of Birmingham in collaboration with Diageo Innovation R&D Commercial Science.

Conflicts of Interest

The authors declare no conflicts of interest related to this work.

Acknowledgements

The authors would like to thank Frank Lynch and David Beverly for their guidance and assistance in the planning and editing of this work.

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Chapter 5- Amino acid and protein manipulation: effect on mouthfeel in beer and model systems

This chapter was prepared as suitable for submission to The Journal of the society of American Brewing Chemists.

Holt , T., & Mills, T. “Amino acid and protein manipulation: effect on mouthfeel in beer and model systems”.

Thomas Holt: methodology, validation, formal analysis, writing (original draft, review and editing).

Tom Mills: methodology, writing (review and editing), supervision, funding acquisition.

Amino acid and protein manipulation: effect on mouthfeel in beer and model systems

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Keywords- tribo-rheology, mouthfeel, protein, beer, brewing

Abstract

Low and no-alcohol beers represent a growing market worldwide, this coupled with a similarly growing market for high protein products represents an avenue for investigation into protein added beers. To investigate the effects these additions may have on mouthfeel properties of commercial and experimental beers this work examines all proteinogenic amino acids as well as two defined proteins in a range of systems of increasing complexity using tribo-rheology. Along with this primary investigation several chemical properties of molecules are examined to elucidate the possible causes of the observed behaviour and the observed system specific effects. This data suggests a possible role for protein addition in the manipulation of mouthfeel properties in beer products, especially within the low and no-alcohol space.

Introduction

With increasing demand from consumers worldwide, brewers have significantly increased offerings of low and no alcohol beers. Historically these products were produced by dealcoholizing finished products or simply by brewing using lower gravity wort (Brányik et al.

2012). The lower starting concentration of simple and complex saccharides from this lower alcohol brewing often resulted in poor customer perception of both flavour and mouthfeel (Krebs et al. 2019). Methods to assess mouthfeel present a challenge to traditional breweries, where tasting panels have been used to assess taste as well as overall perception (Harrison 1970), it has been previously noted that it can be challenging to separate flavour from mouthfeel objectively (Symoneaux et al. 2015; Agorastos et al. 2023). Additionally, the financial and time investment required to have sufficiently competent tasters available when needed presents an additional hurdle, especially for smaller breweries. As such tribology represents an avenue to investigate physical properties of experimental products, reducing reliance on human tasters with benefits for screening large numbers of samples which may not be entirely finalized, but which analysis could still provide valuable insight.

Tribology (Fox et al. 2021) and tribo-rheology (Holt and Mills 2023) have both been utilized for mouthfeel measurement of finished beer products, showing measurable differences between various products, including the low alcohol and standard strength products from the same brewery (Holt and Mills 2023). As of yet, little work has been conducted on the post fermentation manipulation of protein content in the context of mouthfeel, however some data suggests a potential role for proteins and amino acids in tribology friction measurements (data unpublished).

Amino acids and protein content of beer are not commonly manipulated beyond the choice of malt/grain and the quantity used, although the quantity of protein present has long been known to alter brewing outcomes (Smith 1990). This is likely due to lack of demand for products containing specific quantities of amino acids or proteins from this sector of the

beverage market, however with a rise in these products in other sectors, the addition of protein and or amino acids to beer represents a potential expansion area for beer sales, particularly in low and no alcohol products.

Along with the increasing interest in reducing alcohol consumption for health reasons there has been a significant rise in high protein drinks for athletic recovery with evidence supporting their use (Atherton et al. 2017; Niles et al. 2001). This trend has contributed to the appearance of several low alcohol beers with added protein, representing a positive option in terms of health, while also having the flavour expected from a more celebratory product.

While other chemicals have been shown to alter mouthfeel as well as tribological behaviour, many have concerns based on their intrinsic taste profiles. Inorganic sodium salts for example, have been shown to significantly affect lubrication properties (Garrec and Norton 2012) but the levels tested were significantly higher than taste thresholds and as such would make a poor choice for a product which is not expected to have a salt forward taste profile. Similarly, fat content has been seen to play an important role in emulsion tribology (Nguyen et al. 2016) but this is not applicable to non-emulsion based beverages. Dextrin content has classically been considered a significant contributor to mouthfeel of beer (Langstaff and Lewis 1993), although more recently this has been found to not be entirely concentration dependent, with length of the polysaccharides also contributing to the overall perception (Krebs et al. 2019). Dextrins however are relatively energy dense and while offering a slower release profile for energy generation than simple sugars, are not known to provide significant benefits in recovery or injury repair. These factors make the addition of protein and or amino

acids an interesting possibility for tailoring beer products to a new market of health-conscious individuals.

The molecular basis of lubrication is dependent on the speed of entrainment and the axial force present but follow a general pattern with exact speed and friction values varying by system (Hsu 2004). This general scheme is categorised into four regions, hydrodynamic, elastohydrodynamic, mixed and boundary, each determined by its unique tribological properties which determine friction and the molecular basis for lubrication. When speeds are high, lubrication is determined entirely by the bulk properties of the fluid, primarily viscosity (Quinchia et al. 2014) where by more viscous lubricants produce thicker films to separate the two surfaces yielding more effective hydrodynamic lubricant. In this regime there is no physical contact between the two surfaces, just the lubricant films entrained between them, however surface topography will still have a role in the conditions required to maintain this distancing (Priest and Taylor 2000). Once speed is reduced and assuming deformation of either surface is possible elastohydrodynamic features supersede pure hydrodynamic ones. Here a thinner film provides total separation but local deformation of one or both surfaces occurs, due to the thinner films present surface roughness is considered significant for effective elastohydrodynamic lubrication with smoother topographies offering improved lubricity (Priest and Taylor 2000).

Once the hydrodynamic films have partially failed to separate the surfaces, meaning some asperity contact has begun, mixed lubrication is considered to be in effect. Here a mixture of surface deformation, hydrodynamic separation and direct surface contact determine friction. In this regime thin films of adsorbed molecules act as an anti-wear film, providing an

additional layer of material which can be worn away without damaging the original surface. The nature, strength and thickness of these films is dependent upon the molecules being deposited on the surface as well as the physical/chemical properties of the surface itself. With examples seen for simple Van Der Waals and electrostatic interactions (Ismail and Bagheri 2017) to full covalent bonding (Willermet et al. 1995), the nature of the interaction being highly determinant of the forces required to cause its failure. While intact these films act to reduce friction at a molecular level by providing a smoothing effect, as well as slowly yielding and possibly reforming reducing friction by reducing resistance. Further reductions in speed result in the total loss of hydrodynamic lubrication, entering the pure boundary scheme, where the only lubrication is provided by tribofilms and their ability to reduce surface roughness and yield under high friction.

To elucidate the effect of soluble proteins on tribology the classic model proteins bovine serum albumin (BSA) and lysozyme were selected, this selection was based on the availability of pure isolated proteins as well as their solubility in unbuffered solutions, thus avoiding complicating analysis with additional small molecules. For direct comparison on a molecular level 1.5 mM was selected for all initial tests, this value was selected as it is within the solubility limit for of the proteinogenic amino acids in water, for later tests several concentrations were examined, where solubility allowed. These tests were conducted using a range of speeds including the full range considered “normal” for human oral processing (Hiiemae and Palmer 2003) as well as some portion beyond to provide a more complete picture of lubrication within the test systems.

Materials and methods

Water HPLC plus (Sigma Aldrich), L (+) Aspartic acid >98% (Acros Organic), L-isoleucine >98% (Sigma Aldrich), L-(+)-lysine >97% (Tokyo Chemical Industries), L-phenylalanine >99% (Sigma Aldrich), L-leucine >98% (Sigma Aldrich), L-serine >99% (Sigma Aldrich), L-alanine 99% (VWR), L-arginine >98% (Thermo Scientific), L-asparagine >98% (Sigma Aldrich), cysteine, L-glutamic acid 99% (Thermo Scientific), L-glutamine >98% (Fisher Scientific), L-glycine 99%, L-histidine >98% (Thermo Scientific), DL-methionine >99% (Fisher Scientific), L-proline 99% (VWR), L-threonine 98% (Thermo Scientific), L-tryptophan >98% (Sigma Aldrich), L-tyrosine cell culture reagent (Fisher Scientific), DL-valine >99% (Sigma Aldrich)

Maltodextrin 4-7 DE equivalent (6.5 dextrose equivalent mw ~3008)(Sigma Aldrich). Glucose >99% anhydrous (Sigma Aldrich), Fructose >98% (Sigma Aldrich), Sucrose >98% (Sigma Aldrich). Raffinose >99% (Thermo Scientific), bovine serum albumin 99% albumin* (Sigma Aldrich), lysozyme from chicken egg white 93751 U/mg* (Sigma Aldrich), Maltose monohydrate analytical reagent grade >98% (Fisher Scientific). *Where available specific lot analysis information has been included.

A commercial British lager beer with listed alcohol by volume of 4% was obtained from a local supermarket and is referred to as LB4.
A commercial European lager beer with listed alcohol by volume of 0.0% was obtained from a local supermarket and is referred to as LA0.

SafBrew LA-01 low alcohol brewing yeast was acquired from Fermentis.

A defined base medium, Yeast Nitrogen Base with amino acids and ammonium sulphate (Sigma Aldrich) was supplemented with carbon sources to mimic those measured previously (Otter and Taylor 1967) using; maltodextrin 4-7 DE equivalent (Sigma Aldrich), maltose (Fisher Scientific), glucose (Sigma Aldrich), table 1 shows the final concentrations of all components. The media was filter sterilized (0.2 µm) using PES bottle top vacuum filters (Fisher Scientific) and fermented under airlock with 300 ml aliquots in 500 ml conical flasks at 20 ^{o}C (+/- 1 ^{o}C) for 14 days, pitched with the manufacturers recommended rate of 50 g/100 L of wort, representing ~5 x 10^{9} viable cells per litre.

Model beer alcohol by volume was calculated using Equation 2 taken from HM Revenue and Customs excise notice 226 section 30.1-30.3 methodology where-by $ABV = (OG - FG) \times f$, where OG represents original gravity, FG being final gravity and f an arbitrary factor determined previously under Customs and Excise notice 226 in this case f=0.126 as per section 30.3 “Value of factor “f” for various alcoholic strengths” (HM Revenue and Customs Excise Notice 226). Yielding an estimated alcohol by volume of 1.2726%.

Table 4. Composition of defined beer simulation medium pre fermentation. Produced by addition of saccharides/polysaccharides to Sigma Aldrich 51483 Yeast Nitrogen base with amino acids and ammonium sulphate.

Final media composition		g/L
Base media Sigma Aldrich 51483 (6.7g/L)	Ammonium Sulphate	5
	$CaCl_2$	0.1
	Histidine	0.01
	Inositol	0.002
	$MgSO_4$	0.5
	Methionine	0.02
	K_2HPO_4	1
	NaCl	0.1
	Tryptophan	0.02
		ug/L
	p-aminobenzoic acid	200
	Biotin	2
	Boric acid	500
	Calcium pantothenate	400
	$CuSO_4$	40
	Ferric chloride	200
	Folic acid	2
	$MnSO_4$	400
	Niacin	400
	KI	100
	Pyridoxine HCl	400
	Riboflavin	200
	Sodium molybdate	200
	Thiamine hcl	400
	$ZnSO_4$	400
Carbon 10% w/v total to mimic (Otter and Taylor 1967)		g/l
	Fructose	1.106
	Glucose	14.128
	Sucrose	6.143
	Maltose monohydrate	58.968
	Maltodextrin	19.656

Table 5. Basic physical properties of systems used in this work, SG= specific gravity, ABV= alcohol by volume, * this value being calculated by the authors. ABV values for LB4 and LA0 are taken from the manufacturer's declaration.

System	pH	SG (g/ml)	ABV (%)
Water	6.00	1.001	0
Model beer	3.02	1.029	1.2726*
LB4	4.12	1.008	4
LA0	3.90	1.016	0

SYLGARD 184 elastomer kit (Dow Corning) was used to fabricate tribology surfaces as per manufacturer's instructions using 10:1 ratio of polymer to crosslinking agent (w/w) degassed under vacuum and cured at 100 ^{o}C for 35 minutes in 3D printed resin moulds. Each surface was used for a single replicate before being replaced.

All solutions were produced weight per volume using grade A volumetric flasks (Fisher Scientific).

All solutions and beers were thoroughly degassed by sonication to avoid agitation from dissolution of amino acids or proteins altering behaviour. 0.22 µm poly-ethersulfone syringe filters (SLS) were used to filter any particulates from the tested samples.

Yeast cells were counted and assessed for vitality using 0.01% methylene blue (Alfa Aeasar) in 0.1% disodium citrate (Fisher Scientific), which was mixed 1:1 with cell suspension before manual assessment under 20x magnification using a cell counting chamber (Hirschmann). Cells which took up the dye were considered non-viable for pitch rate calculations.

Instrumentation

Discovery hybrid rheometer HR-1 (TA Instruments) with 3 balls on plate top geometry (aluminium) (TA Instruments). Bottom sample holder was a locally produced 3D printed resin cup (STL file included in supplementary information). The axial force was fixed at 1 N (+/- 0.1 N) while sliding speed was varied from 150 mm/s to 0.15 mm/s with 10 data points recorded per decade. This system was used to produce Stribeck curves for test samples, the data from which was then used to produce difference from solvent graphs by subtracting values obtained from the solvent system from samples based on 3 replicates using a fresh PDMS disk for each.

Density measurements were conducted using a Densito Handheld densitometer accuracy +/- 0.001 g/ml, measurements were taken in triplicate and averaged.

pH readings were conducted using a Metler Toledo SevenCompact pH and Ion meter, with a Metler Toledo InLab Expert Pt1000, calibrated with pH 4, 7 and 10 standard buffers (Fisher Scientific).

Results

Coefficient of friction measurements collected at 1N axial force between the sliding speeds of 150 and 0.15 mm/s were used to produce solvent subtracted coefficient of friction plots, showing the change in friction from just the solvent for that sample, Figure 16 shows these values for all 20 proteinogenic amino acids, BSA and lysozyme measured in water.

Immediately it can be observed that amino acids do alter friction behaviour in water, with many of the tested molecules showing significant changes in friction at various speeds. Figure 16A shows the results obtained from arginine, histidine, lysine, aspartic acid and glutamic acid, here aspartic acid provides statistically significant reductions ($p<0.05$) in friction compared to water alone at every speed tested, while arginine demonstrates a smaller change to friction values, while still remaining significant at almost all test points, except those between 15 and 9.5 mm/s. Histidine however only occasionally induces a change which is significant, indicating it has a much lower impact on either hydrodynamic effects or boundary lubrication.

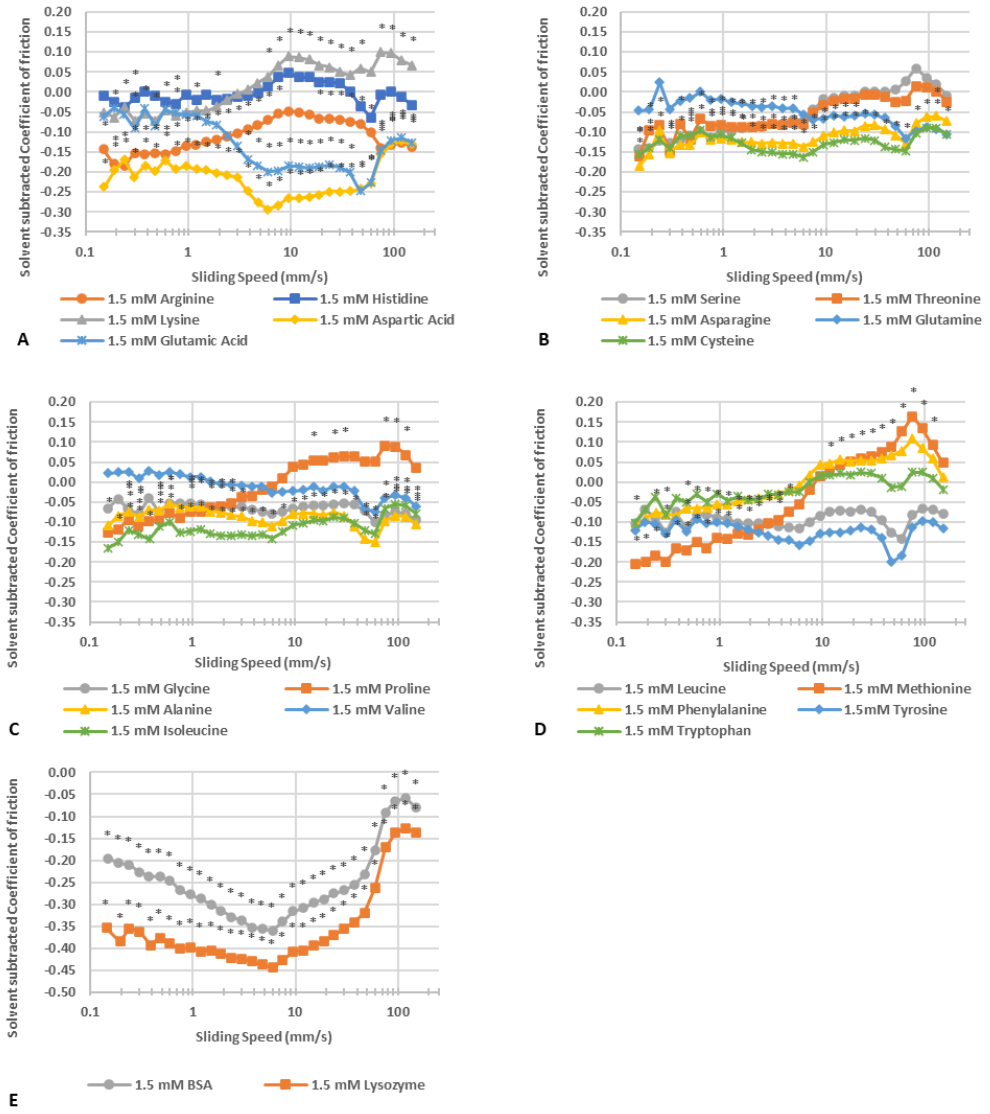


Figure 16. Solvent subtracted coefficient of friction over 0.15 mm/s to 150 mm/s with 1N axial downforce for solutions of proteinogenic amino acids in water (A-D) or bovine serum albumin and lysozyme (E). All with a concentration of 1.5 mM. * marks denote a statistically significant (p=<0.5) difference between coefficient of friction values and ultra-pure water tested alone.

In contrast, lysine shows a different profile to the other four, significantly increasing friction in the high-medium speed range, while providing significant reductions in friction at low-very low speeds. This would indicate that lysine has a negative effect on hydrodynamic lubrication, potentially causing mixed regime contact earlier than in the other tested samples, or that viscosity is altered sufficiently to cause this weakening of the hydrodynamic

effects, while then being capable of forming successful boundary lubrication films at lower speeds. Although previous work has indicated that significantly higher concentrations of amino acids would be required to noticeably alter viscosity (Daniel and Cohn 1936), with tested values often several orders of magnitude higher than utilized here. Again, differing to lysine, glutamic acid produced significant reductions only in very high-medium speeds, indicating it is more effective in hydrodynamic lubrication based effects than boundary.

Figure 16B contains the solvent subtracted coefficient of friction over speed graphs for serine, threonine, asparagine, glutamine and cysteine, here some similar profiles to Figure 1a can be seen, although the absolute changes in friction are smaller for most of the tested molecules in this group. Serine for example shows no significant changes at any speed, indicating it is unable to alter hydrodynamic or boundary effects sufficiently to alter the overall behaviour. Threonine demonstrates a novel behaviour, showing consistently significant reductions in friction, but only at speeds lower than 5 mm/s, this is indicative of a molecule with little to no effect on bulk properties but able to provide strong boundary lubrication, likely through tribo-film formation. A similar behaviour is seen with asparagine, except it exerts some friction reduction into the mixed regime area, beginning reductions in friction from 18 mm/s. Glutamine, similarly to histidine, shows very limited friction changes suggesting it is not effective at forming tribo-films or altering bulk behaviour. Cysteine, similarly, to aspartic and asparagine shows a significant reduction in friction at almost all test speeds.

Figure 16C shows the results of glycine, proline, alanine, valine and isoleucine. Within these, glycine and alanine both show a broadly similar pattern initially yielding significant reduction

in friction at very high speed, before losing the effect then regaining a lubricity benefit at medium-very low speeds. Proline shows some increases in friction at higher speeds, while reducing it at very low speed, similar to, but to a greater extent than serine. While valine provides no significant changes in friction at any speed and isoleucine improves lubricity at all speeds except 0.15 mm/s.

The final group of amino acids (Figure 16D) show some novel patterns, with methionine yielding the largest increase in friction at high speeds, while then also providing a noticeable reduction in friction as speed is reduced, crossing over the no change region at 9.5-5.9 mm/s. Phenylalanine exhibits a similar profile to methionine, but is not significant at any speed, which could suggest the two molecules share some partial mechanism of action, but it is more pronounced in methionine. Meanwhile tyrosine and tryptophan display a very similar profile, with no significant differences from water with tyrosine and only two data points with tryptophan indicating they do not significantly alter lubrication in water based systems.

Data for the proteins BSA and lysozyme are shown in Figure 16E. These show significant reductions in friction at all test speeds, although lysozyme yields a numerically greater reduction than BSA. The pattern of friction reduction is not shared with any of the amino acids, being most similar to aspartic acid, but is not closely related.

From this data amino acids can be roughly characterised by their tribological behaviour based on the alteration of friction at roughly divided speed ranges. High speed, where hydrodynamic contributions are more important, medium speed, where both hydrodynamic and boundary interactions occur and low speed where only boundary interactions determine

behaviour. Once separated by simply assigning a +1 to samples which increase friction significantly, a 0 to those which neither increase or decrease friction and a -1 to those which significantly reduce it a large number of samples can be screened and compared. Table 3 shows this tabulated for all twenty amino acids, where a sample showed some significant changes but not uniformly, the behaviour shown by 50% or greater for the region was used to assign the value.

An interesting immediate observation is that none of the aromatic amino acids had significant effects on friction in any region, phenylalanine, tyrosine and tryptophan were among 6 amino acids with no observed effects on friction. To investigate potential causes for this observation the physiochemical properties of the amino acids were plotted against their friction coefficients at three different speeds, representing 1 point from each decade tested, 95, 9.5 and 0.95 mm/s were chosen as these points fall within the high, medium and low speed regions and represent a broad but still simplified method to compare samples (Figures 2 and 3).

Table 6. Simplified friction changes table for proteinogenic amino acids in water, values of 0 indicate that greater than 50% of points within that region were not significantly different from water ($p<0.05$ for significance), a value of 1 indicates that greater than 50% of points within that region significantly increased coefficient of friction compared to pure water, while a value of -1 indicates that greater than 50% of points within that region significantly reduced coefficient of friction compared to pure water.

Amino acid	High Speed	Medium Speed	Low Speed
Glutamic	-1	-1	0
Arginine	-1	-1	-1
Histidine	0	0	0
Lysine	1	0	-1
Aspartic acid	-1	-1	-1
Serine	0	0	0
Threonine	0	0	-1
Asparagine	0	-1	-1
Glutamine	0	0	0
Cysteine	-1	-1	-1
Glycine	0	0	-1
Proline	1	0	-1
Alanine	-1	0	-1
Valine	0	0	0
Isoleucine	-1	-1	-1
Leucine	0	0	-1
Methionine	1	0	-1
Phenylalanine	0	0	0
Tyrosine	0	0	0
Tryptophan	0	0	0

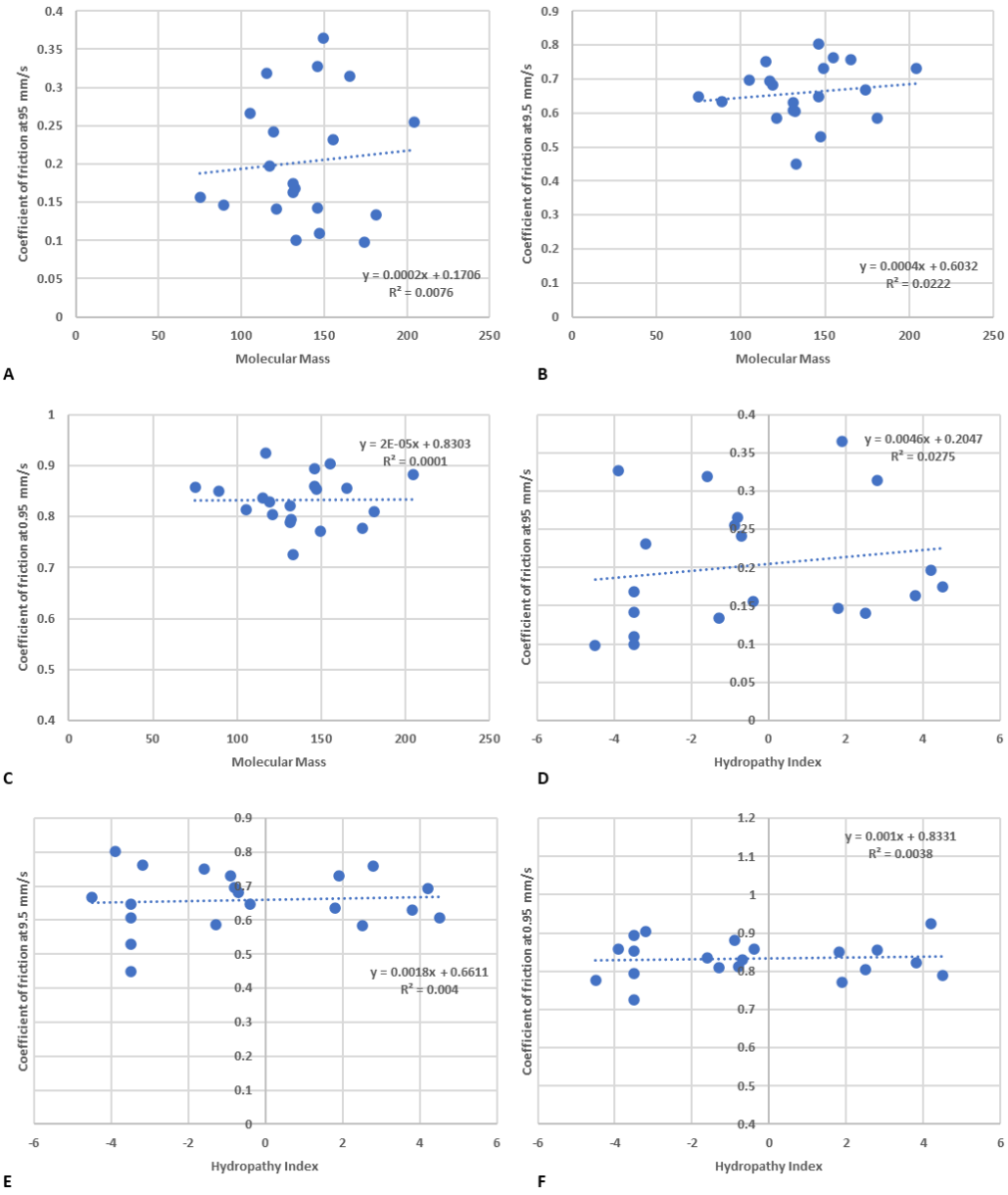


Figure 17. Correlation plots of coefficient of friction vs molecular mass (A-C) and hydropathy index (D-F) of 20 proteinogenic amino acids at 1.5 mM in water, at three sliding speeds, 95 mm/s (A and D), 9.5 mm/s (B and E) and 0.95 mm/s (C and F). R^2 is given as a measure of fit quality between the two variables.

With all 20 amino acids considered no significant correlations were found between friction and molecular mass (Figure 17A-C), with the strongest observed at 9.5 mm/s with an R^2 value of 0.0222. This indicates that molecular mass alone is not a significant factor in friction behaviour at any tested speed and suggests that other properties are likely more important

to tribological behaviour. As such hydropathy index was examined. This property is a computationally assigned value commonly used to assist in identifying membrane domains of proteins but provides a numeric value for hydrophobicity specific to amino acids to allow comparison (Mitaku et al. 2002). When coefficient of friction is plotted against hydropathy index, similarly to molecular mass no strong correlations were observed (Figure 17 D-F), with the strongest correlation at 95 mm/s being R^2= 0.0275. This lack of correlation is possibly due to the diversity of polar groups within amino acids, while all have a carboxylic acid and an amine group the variety of side chains is significant. This diversity means that while overall two given amino acids may have similar polarity by this index, their chemical properties may be significantly different. For example glutamine and aspartic acid both have a hydropathy index of -3.5 (Mitaku et al. 2002) while one is positively charged in solution and the other negatively, this would be expected to significantly alter the properties of the molecules based not on their hydrophobicity but their ability to interact with surfaces.

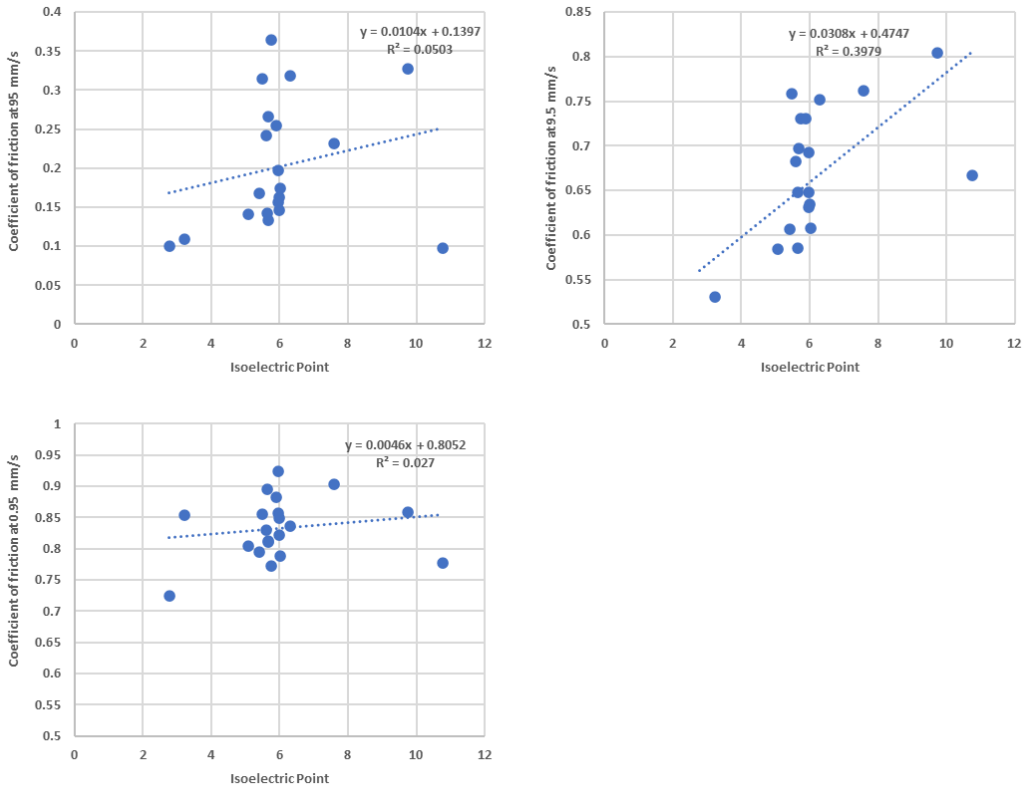


Figure 18. Correlation plots of coefficient of friction vs isoelectric point (A-C) and contact angle on PDMS (D-F) of 20 proteinogenic amino acids at 1.5 mM in water, at three sliding speeds, 95 mm/s (A and D), 9.5 mm/s (B and E) and 0.95 mm/s (C and F). R^2 is given as a measure of fit quality between the two variables.

Several measures could be utilized to quantify dissociation/charge changes, given that all the amino acids contain an amine and carboxylic acid group isoelectric point was utilized rather than pKa, due to it providing a more representative value for the whole molecule in unbuffered solutions than the dissociation values of the acidic or basic groups. Figure 18 A-C shows the results of coefficient of friction plotted against isoelectric point. As with molecular mass and hydropathy index at 95 mm/s a very poor correlation between the two variables is observed, this does however change at 9.5 mm/s, where a moderate correlation of 0.3979 is seen between isoelectric point and friction. At 0.95 mm/s a very weak correlation of 0.027 was observed. The trends demonstrated are positive, such that increased isoelectric points tend to yield higher friction coefficients, making basic amino acids less effective lubricants than more acidic ones in general. Although, most amino acids have an isoelectric point between 5 and 6 (Liu et al. 2004) there is a large group of molecules where this trend is inaccurate being primarily derived from the most acidic and most basic molecules. This finding does suggest that isoelectric point is only a real consideration when it is outside of the range of 5-6, since this experiment was conducted in water it could indicate that this solution was not acidic or basic enough to cause significant partition in the populations of positively and negatively charged molecules. Given that a beer product would be expected to have a pH between 4 and 5 (Coote and Kirsop 1976), this change of environment would be expected to cause significant changes to the ionization of the amino acids and thus their behaviour. To investigate this a model low alcohol beer was produced from defined ingredients (see methods).

The fermented media was found to have a final pH of 3.02 with a final gravity of 1.029 g/ml from a starting gravity of 1.039 g/ml. This media was used as the sample solvent for a repeat

of the water-based experiments from Figure 16. Immediately it was found that while tyrosine was sufficiently soluble in water to be used, its solubility in this media was very limited and so tyrosine was not tested. Initially this model beer was compared with water as well as two commercial lager beers LB4 and LA0, representing a 4% alcohol by volume product and a 0% alcohol product. Figure 18A shows the Stribeck curves generated from these samples. From this data it can be seen that the model beer has similar lubrication properties to a commercial 0% beer with somewhat higher friction during low speeds than is observed with the alcoholic beer. It is also apparent that all three tested samples are significantly better lubricants than pure water, showing markedly lower friction at all speeds. Having established that this model beer is representative of commercial products the previously tested amino acids were dissolved in it and Stribeck curves generated in the same fashion as with water in Figure 16. The results demonstrate a significant departure from those seen in water: amino acid mixtures, primarily in that the addition of amino acids at 1.5 mM to this analogue is seen to increase friction in all cases, except for a small number of data points for four amino acids or proteins. The friction changes are primarily found at speeds below 100 mm/s, indicating the effect on hydrodynamic lubrication is relatively limited when compared to the changes to boundary lubricity.

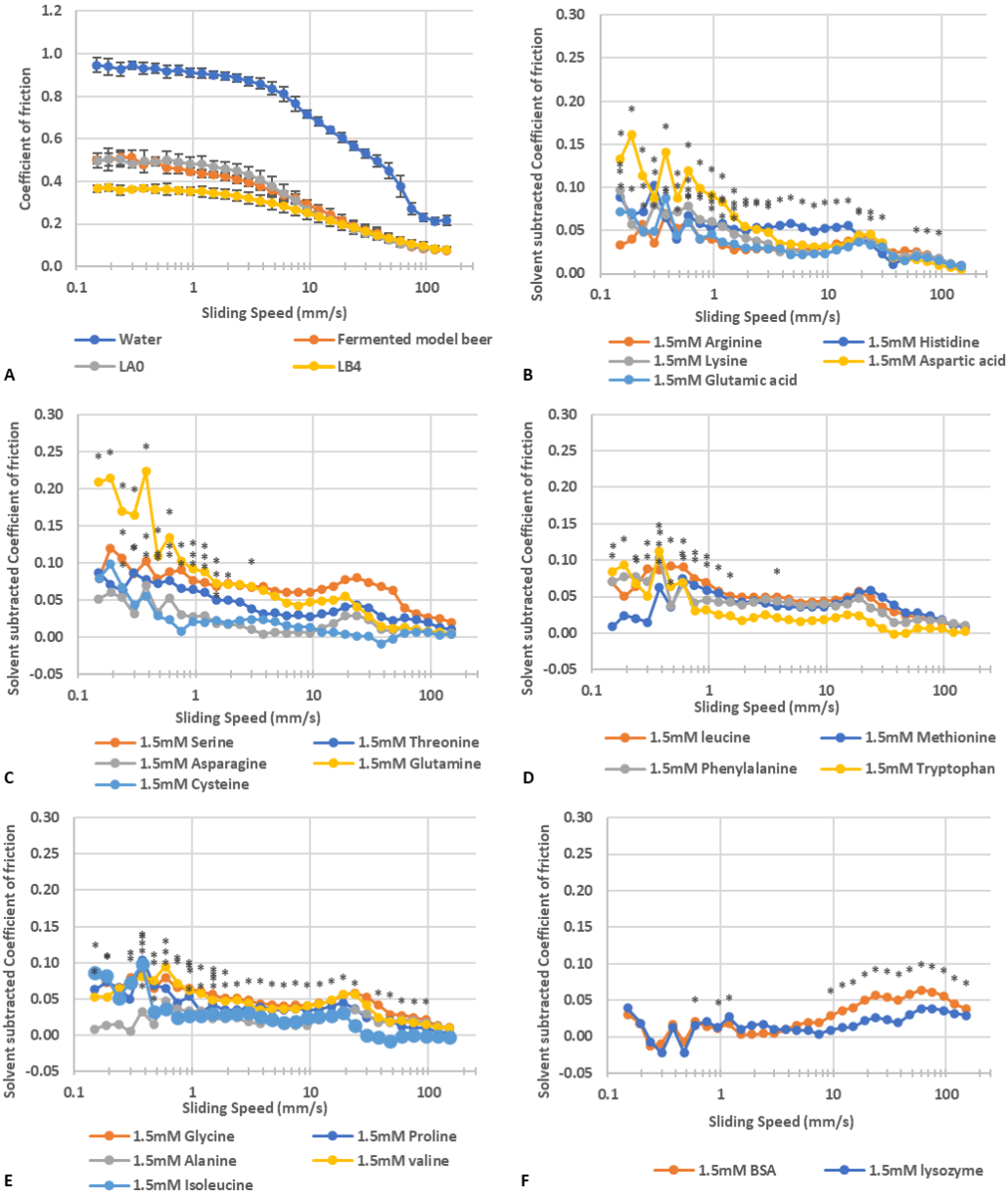


Figure 19. (A) Stribeck curves generated over 150 mm/s to 0.15 mm/s with 1N axial downforce for water, a defined simulated beer (see Table 1) and two commercial beers, 0% alcohol LA0 and 4% alcohol LB4 without additions. Error bars denote 1 standard deviation.

Solvent subtracted coefficient of friction over 0.15 mm/s to 150 mm/s with 1N axial downforce for solutions of proteinogenic amino acids in defined simulated beer media post fermentation (B-E) or bovine serum albumin and lysozyme (F). All with a concentration of 1.5 mM. * marks denote a statistically significant (p=<0.5) difference between coefficient of friction values and the test media when examined alone. Tyrosine was omitted due to low solubility.

The exact mechanism for this increase in friction is not fully explained by this data, but could be hypothesised to be related to stability of tribofilms, in the water and amino acid samples there are very few suitable molecules other than the amino acids present to produce any kind of smoothing film. Where as in the relatively complex fermented media the abundance of saccharides, particularly given the low attenuation of this analogue provides a strong capacity for tribofilm formation, hence its markedly lower friction than water alone. With the addition of the amino acids they may provide some level of competitive binding for the sugar molecules adsorption to the surfaces thus disrupting the boundary lubricity without significantly altering the bulk properties of the fluid and so leaving hydrodynamic lubricity largely unchanged. While at most speeds for most samples the effect on lubricity is negative, the absolute values of increase are relatively limited for most samples, especially at high-medium speeds. Within the range generally considered relevant to human oral processing (2.10 mm/s to 32.43 mm/s) (Hiiemae and Palmer 2003) most amino acids show very small changes in friction often in the range of +0 to +0.05 coefficient of friction, while showing several times that increase at very low speeds (below 1 mm/s).

Using the coefficient of friction values obtained from the fermented media correlations were again plotted for molecular mass, hydropathy index (Figure 17) and isoelectric point (Figure 18). These, similarly, to those plotted with water and amino acids do not show significant correlations for any of the variables with an R^2 value of 0.3052 for hydropathy index at 9.5 mm/s being the strongest correlation. It is however noted that while most are still poorly correlated, the correlations are several orders of magnitude greater than those seen with the water-based samples.

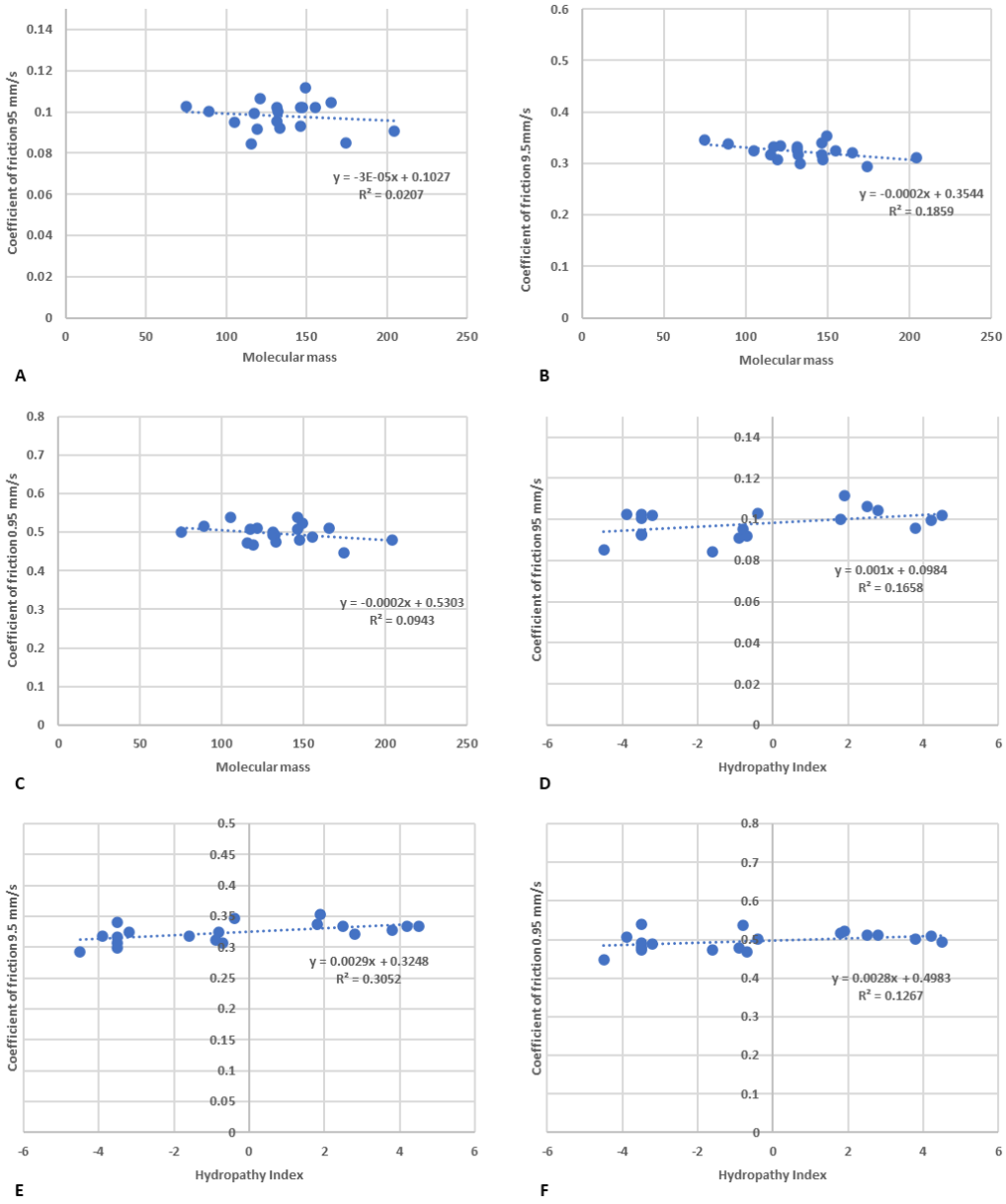


Figure 20. Correlation plots of coefficient of friction vs molecular mass (A-C) and hydropathy index (D-F) of 20 proteinogenic amino acids at 1.5 mM in defined beer analogue, at three sliding speeds, 95 mm/s (A and D), 9.5 mm/s (B and E) and 0.95 mm/s (C and F). R^2 is given as a measure of fit quality between the two variables.

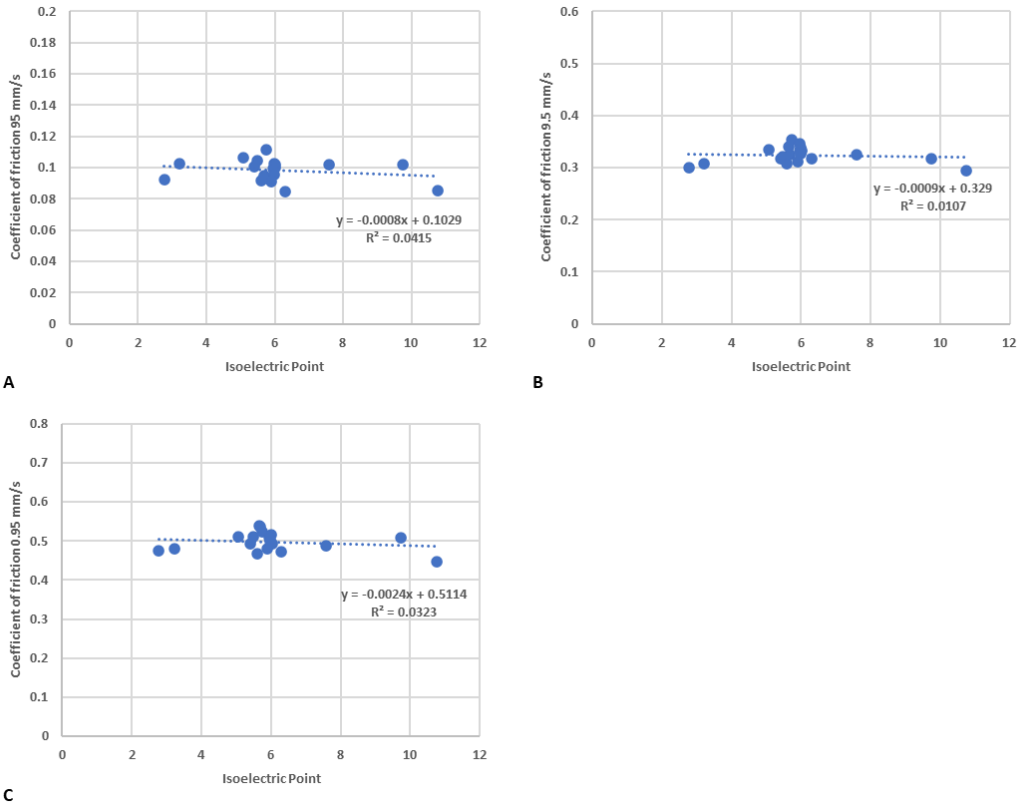


Figure 21. Correlation plots of coefficient of friction vs isoelectric point (A-C) of 20 proteinogenic amino acids at 1.5 mM in defined beer analogue, at three sliding speeds, 95 mm/s (A and D), 9.5 mm/s (B and E) and 0.95 mm/s (C and F). R^2 is given as a measure of fit quality between the two variables.

Medium	Property	R^2 95 mm/s	R^2 9.5 mm/s	R^2 0.95 mm/s
Water	Molecular mass	0.0076	0.0222	0.0001
	Hydropathy index	0.0275	0.0040	0.0038
	Isoelectric point	0.0503	0.0398	0.0270
Model beer	Molecular mass	0.0207	0.1859	0.0943
	Hydropathy index	0.1658	0.3052	0.1267
	Isoelectric point	0.0415	0.0107	0.0323

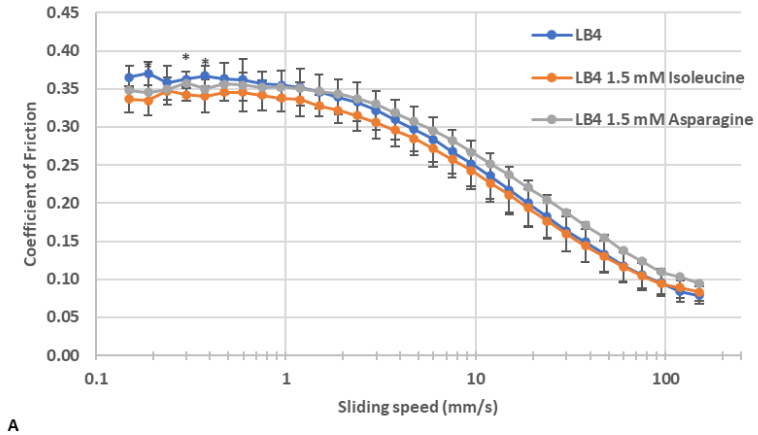
Table 7. Summary of correlation analysis between various properties and coefficient of friction at 95, 9.5 and 0.95 mm/s. R^2 values are given to four decimal places, red text indicates a correlation is negative (a greater value for the property yields lower friction), while green text indicates positive correlation (a greater value for the property yields higher friction).

Table 7 shows a summary of the correlations observed between coefficient of friction and molecular mass, hydropathy index and isoelectric point, both in water and model beer systems. It also demonstrates the nature of the correlation, either positive or negative with respect to the property vs friction.

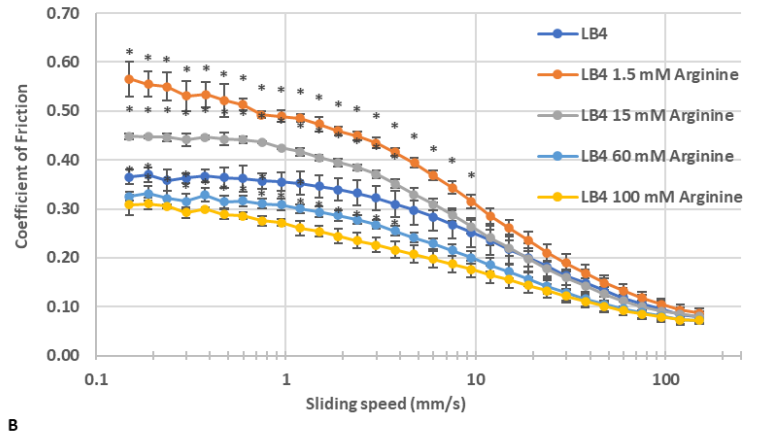
Having examined the effect of exogenous amino acids and proteins in model beer and water, those which demonstrated reduced friction were selected for use in commercial beer products. The selection process for amino acids also included selection based on their flavour, as for consideration for use in an actual product adding a significant taste is a concern, for example cysteine and methionine are known for having a sulphurous taste (Solms 1969) and would likely not be acceptable to consumers at high concentrations. Using friction behaviour and to a lesser extent taste the amino acids arginine, asparagine, aspartic acid, glutamic acid and isoleucine were selected as well as both proteins BSA and lysozyme. BSA and lysozyme would not be considered suitable additives for commercial products, but due to low availability of appropriate purified proteins as well as the low purity generally associated with crude extracts, these were utilized. To investigate the feasibility of adding these molecules to beer several concentrations were attempted of each. Protein concentrations were limited to 1.5 mM and below due to solubility limits, while amino acids were tested at 1.5, 15, 60 and 100 mM where solubility allowed.

It was immediately observed that asparagine and isoleucine were only soluble in one of the beers and only at the 1.5 mm concentration previously utilized as such data for asparagine and isoleucine is only presented for LB4. Figure 22A shows the results of asparagine and isoleucine tribology when dissolved in LB4, from this data it appears that even the highest

concentration that is soluble provides very limited effect on friction behaviour once a commercially relevant beer is considered, this is likely due to the further complexity of the system overcoming the properties of these molecules given their maximum concentration. Arginine however was found to be sufficiently soluble in both beers to test the full 1.5-100 mM range described previously, Figure 22b and 22C show the Stribeck curves generated. Contrary to findings from both water and the model beer, in commercial products 1.5 mM arginine yields a significant increase in friction at low and very low speeds for both alcoholic and non-alcoholic beers. This is unexpected, but like other amino acids in the model system, this different behaviour is likely due to specific molecular composition differences between the commercial products and the analogue. The region of effect is telling as to the likely cause, given the low speeds involved the effect is most probably related to boundary lubrication interruption, either from the arginine interfering with the existing tribofilms directly by competing for binding spots and providing a less uniform surface, or by binding to the already present films but due to the relatively low concentration forming an inconsistent additional layer, adding to rather than reducing aporetic contact.



A



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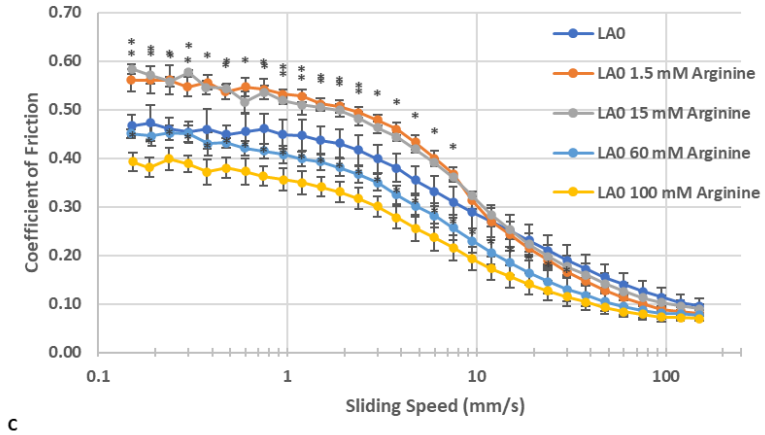
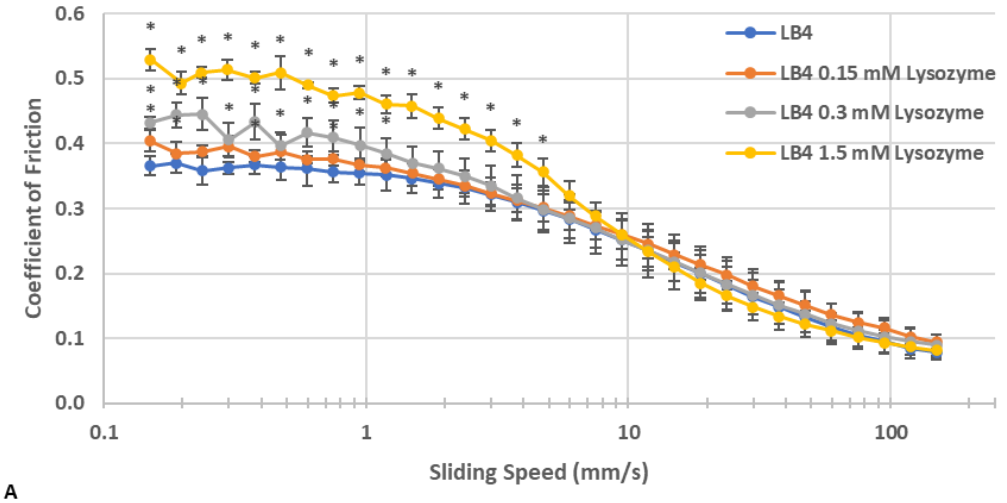


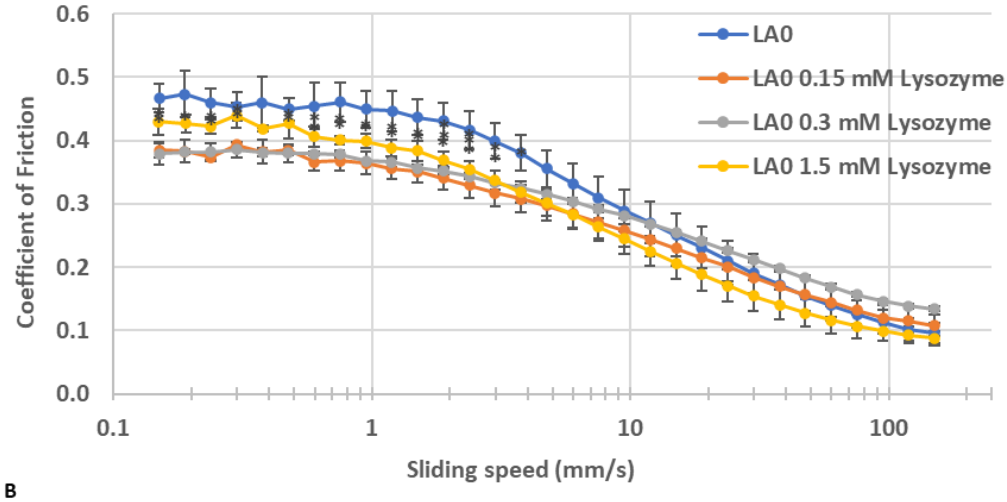
C

Figure 22. Stribeck curves generated over 150 mm/s to 0.15 mm/s with 1N axial downforce for (A) LB4 with added isoleucine or asparagine, (B) LB4 with varied concentrations of arginine, (C) LA0 with varied concentrations of arginine. * marks denote a statistically significant (p=<0.5) difference between coefficient of friction values and the beer when measured without additions. Error bars denote 1 standard deviation.

An increase in arginine concentration to 15 mM causes a divergence between the behaviour exhibited by the alcoholic and non-alcoholic beer, while both show significantly increased friction above the base product, the alcohol containing beer also demonstrates a clear difference from 1.5 mM. This difference between concentrations is not observed in the low alcohol beer system, this shows the concentration for lubrication effects is not constant and is indeed based on the system contents. A system dependent difference is also seen when 60 mM arginine is considered, here a small but significant reduction in friction is observed at low and very low speeds in the alcoholic beer, while a non-significant reduction is seen more generally at speeds between 1 mm/s and 40 mm/s in the non-alcoholic beer. Finally, once a concentration of 100 mM is reached both test beverages show a significant reduction in friction, although absolute values are still different, indicating that arginine is not the only factor determining the lubricity even at this concentration. Overall, this pattern supports a hypothesis where small quantities of arginine are disruptive to the original boundary lubrication, but with increased concentration can come to supplant it, yielding a more effective lubrication surface. The clear system specific differences support the idea that even two different examples of the same type of beer will not behave identically with the addition of exogenous molecules as the exact composition provides a unique lubrication environment which can be disrupted, aided, or replaced depending on the additive and its concentration, with particularly pronounced effects at low speeds.



A



B

Figure 23. Stribeck curves generated over 150 mm/s to 0.15 mm/s with 1N axial downforce for (A) LB4 with various concentrations of lysozyme added, (B) LB4 with varied concentrations of lysozyme added. * marks denote a statistically significant (p=<0.5) difference between coefficient of friction values and the beer when measured without additions. Error bars denote 1 standard deviation.

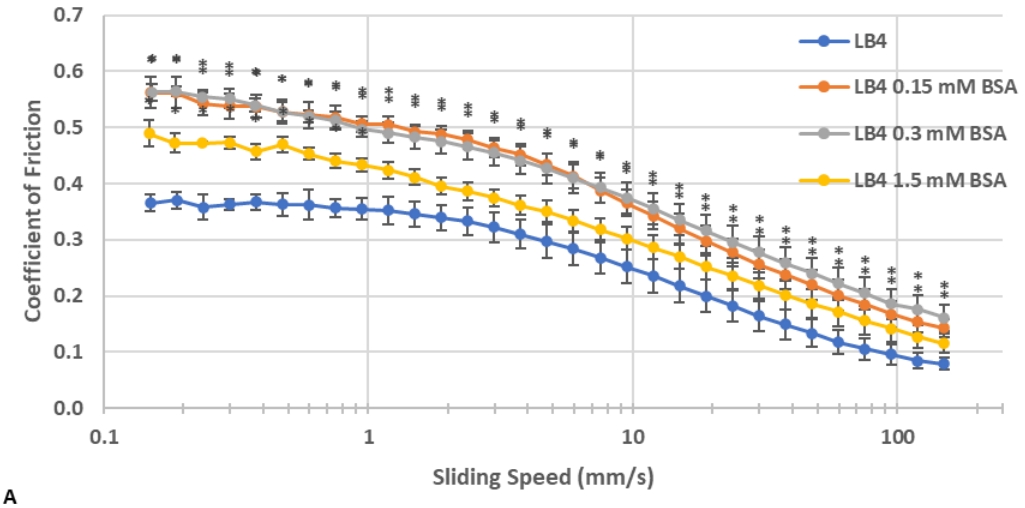
Addition of lysozyme was shown to be highly effective at increasing lubricity in water and ineffective in model beer. Due to the high molecular mass of proteins concentrations greater than 1.5 mM were not viable due to solubility, as such concentrations of 0.15, 0.3 and 1.5 mM were tested. Figure 8 shows the resulting Stribeck curves for both LA0 and LB4. When the alcoholic beer is considered 1.5 mM lysozyme provides a notable increase in friction at speeds below 4.7 mm/s, this effect was not observed in water or model beer systems and is

also not observed in the commercial no-alcohol beer. With the system specificity of this observation, it suggests a possible link between the observed friction increase and alcohol content, although other compositional differences could also be causative. Similarly to 1.5 mM, 0.3 mM samples also demonstrate increased friction above the original product although the increase is numerically smaller it is still significant over part of the speed range seen with the higher concentration. This would suggest the mechanism is concentration dependent supporting the hypothesis of interference in a competitive mechanism, rather than uncompetitive, it also indicates that a significant number of molecules are required to produce the effect as it is noticeably diminished once the concentration is lowered to 0.15 mM (representing 2.145 g/L protein).

Upon addition to the commercial non-alcoholic beer a different pattern is observed, Figure 23B shows the generated Stribeck curves. Here 1.5 mM lysozyme does not significantly alter friction, although it does show some non-significant decreases in friction over a range of medium to low speeds, this contrasts with the significant increases seen in the alcohol containing beer. Similarly to the alcoholic beer, concentrations below 1.5 mM lysozyme show lower friction forces than 1.5 mM, however with the non-alcoholic samples this equates to friction levels which are significantly lower than those found in the original product when speeds are under 3.7 mm/s. Interestingly in this instance no significant difference is observed between 0.3 and 0.15 mM lysozyme which indicates a relatively narrow concentration range for reductions in friction and suggesting that the saturation point for the positive friction effects is close to double the normal concentration for protein in beer ~1% (Abernathy et al. 2009). Lysozyme represents a relatively small mass for a protein at ~14,300 Da, this contrasts with bovine serum albumin, which has a molecular mass of ~66,000 Da corresponding to a

medium-large sized protein. Beer has been shown to contain a wide range of protein sizes and structures (Iimure and Sato 2013), to further investigate proteins effects on friction behaviour BSA was added to both LA0 and LB4 at the same molar concentration as lysozyme. Due to the significantly increased molecular weight these correspond to much higher values for protein when considered in weight/volume but were found to be stable in solution.

Figure 24 shows the Stribeck curves generated using varied concentrations of BSA in both LA0 and LB4. This data shows a clear increase in friction in both systems upon the addition of BSA, such that lower concentrations increase friction more the higher concentration. BSA shows a more significant absolute difference than lysozyme, where a large gap is visible between the behaviour of even the best performing protein addition compared to the unaltered product. It is interesting to note that similarly to lysozyme in both samples 0.15 and 0.3 mM protein produces a similar profile, while 1.5 mM begins to show differences. This is again indicative that with lower levels of protein a poorly lubricating regime is formed between the original systems innate behaviour and the additional proteins in a competitive manner. From this data even 1.5 mM (99 g/L) is not sufficient to improve upon the original lubrication properties within the alcoholic beer, while in the non-alcoholic beer lysozyme was able to significantly reduce friction, indicating system specific interactions even between BSA and lysozymes effects.



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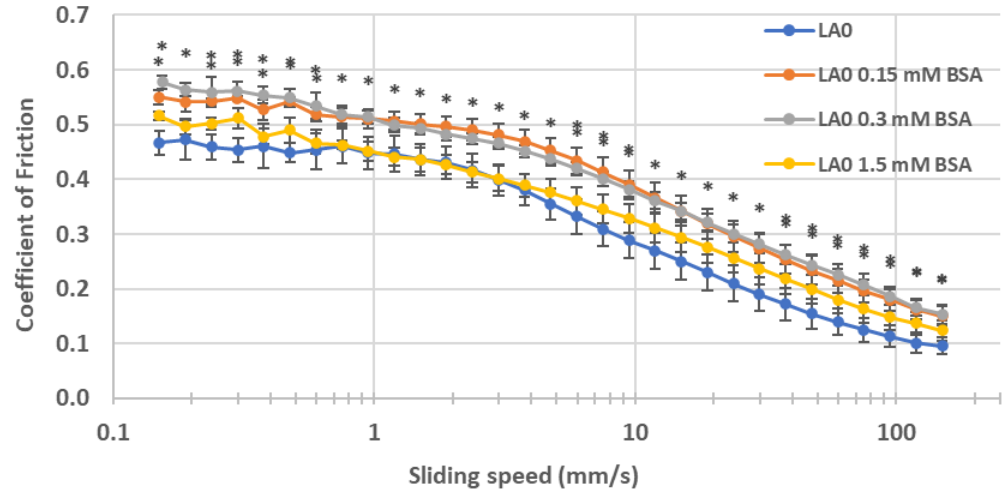

B

Figure 24. Stribeck curves generated over 150 mm/s to 0.15 mm/s with 1N axial downforce for (A) LB4 with various concentrations of bovine serum albumin added, (B) LB4 with varied concentrations of bovine serum albumin added. * marks denote a statistically significant (p=<0.5) difference between coefficient of friction values and the beer when measured without additions. Error bars denote 1 standard deviation.

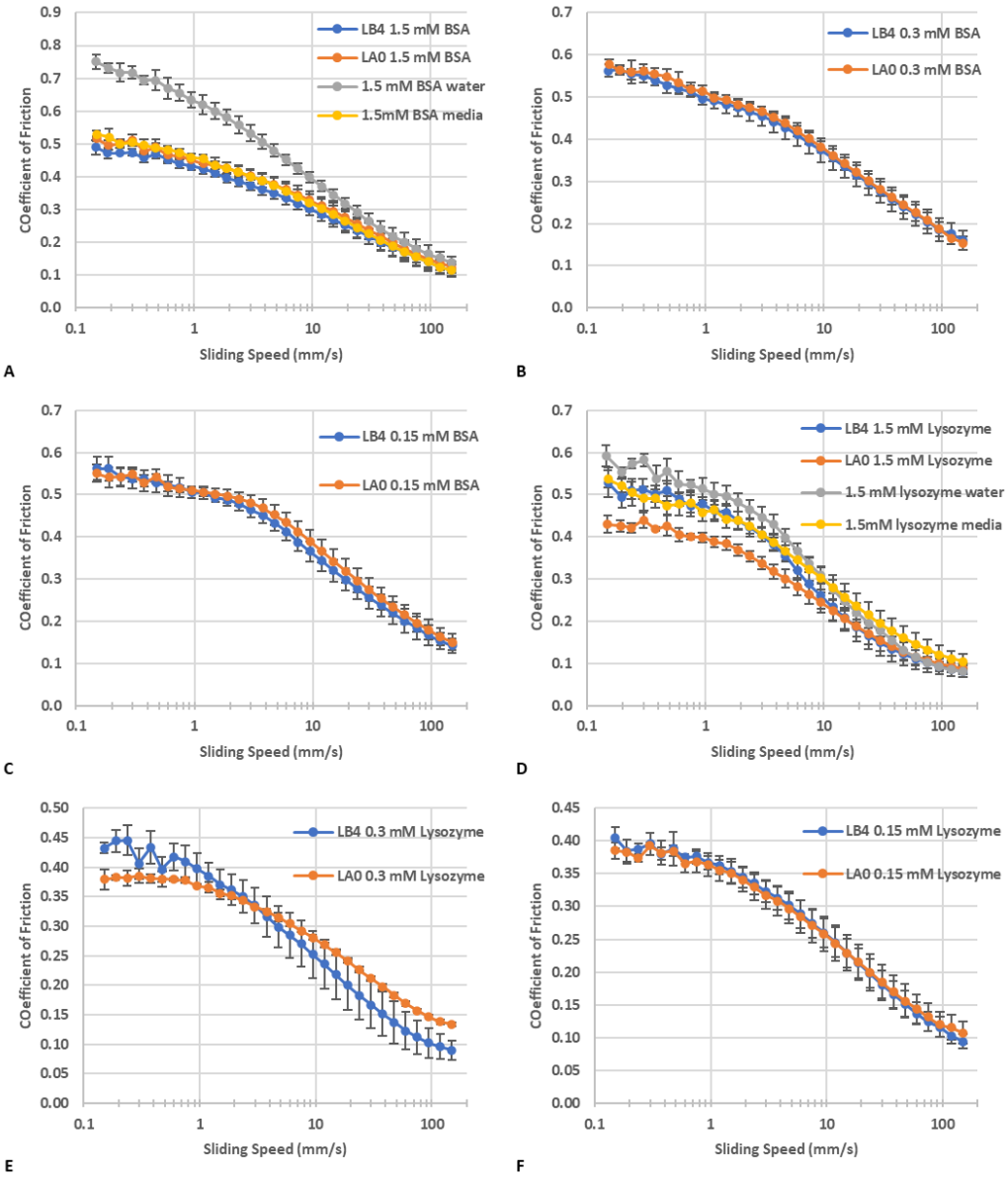

Figure 25. Stribeck curves generated over 150 mm/s to 0.15 mm/s with 1N axial downforce for (A) LB4 with various concentrations of bovine serum albumin added, (B) LB4 and LA0 with 0.3 mM bovine serum albumin added, (C) LB4 and LA0 with 0.15 mM bovine serum albumin added, (D) 1.5 mM lysozyme behaviour in all tested systems, (E) LB4 and LA0 with 0.3 mM lysozyme added, (F) LB4 and LA0 with 0.15 mM lysozyme added. Error bars denote 1 standard deviation.

Upon examination of the absolute figures for friction, it was noted that they are remarkably similar between the systems when the same concentration of protein is examined. Figure 25 shows the Stribeck curves for the same protein concentration but with different beer systems. This high level of similarity suggests that the protein is the dominant factor in determination of friction, even at these lower concentrations and the comparative differences between the effects seen initially are actually artefacts from comparison to the original beer. When in reality, the determining factor for friction behaviour is already the protein concentration even at the lowest 0.15 mM concentration. It was still visible that BSA and lysozyme behave differently even at the same concentration however, reinforcing that the lubricating molecule is still relevant rather than the observed effects being due to just concentration. It should still be noted that the behaviour in pure water of 1.5 mM protein was not analogous to that seen in media, LA0 or LB4, indicating that the system does play some role but that it is more complex than initially thought.

A possible explanation for this observation is the pH difference between a pure water plus protein system and that of a beer product. The significantly lower pH of beers compared to water would be expected to alter the properties of the protein, given the isoelectric point of BSA is between 5.1 and 5.5 (Peters 1985) while lysozymes' isoelectric point is approximately pH 11 (Proctor et al. 1988). This would result in very different charge behaviour for both proteins at the measured pH of the beer samples when compared to water, those being LA0 3.90, LB4 4.12 and fermented media 3.02 (Table 5). The seemingly system agnostic behaviour of BSA is contrasted with lysozyme (Figure 25), where its behaviour is similar in alternative non-water systems, it is far more heterogeneous than BSA. This would not be expected if the isoelectric point is the deciding factor, given that these systems are all several pH units lower

than lysozymes' isoelectric point of 11, meaning effectively all molecules should be in the same charge state (positive). While BSA would be expected to have a mixture of positive and uncharged species, skewing towards a higher distribution of positively charged residues, this difference in isoelectric point could explain the alternative behaviour of BSA in water, where the pH is above its isoelectric point by approximately 0.5-1 unit. Here the uncharged and negatively charged proteins would interact significantly differently to the largely positive charges found in the beer systems, where pH is ~1 unit below the isoelectric point. It is also possible this charging results in a conformational change which has been observed previously in other proteins (Zand et al. 1971) or a confirmational change induced by the low ionic concentration of ultra-pure water, as ionic strength has been seen to alter internal and surface residue behaviour in proteins (Kříž et al. 2013).

Despite these hypotheses appearing reasonable with respect to BSA there is little to support their validity when lysozyme is considered, this could be due to salt contamination within the purified proteins themselves, as no ion content was available for these products or simply that lysozyme is less vulnerable to these changes and so its behaviour is not governed by them to the degree seen with BSA. While BSA and lysozyme show system agnostic friction behaviour at 0.15 mM the absence of this phenomena at other concentrations indicates we are unable to adequately explain the processes and further investigation, ideally with a greater range of proteins would be required.

Conclusions

The data presented here demonstrates a role for amino acids and proteins in mouthfeel as well as examining the complexity of attempting to predict tribo-rheology results even within a single class of molecules. It also demonstrates the challenges associated with altering lubricity in commercial beer products with applications to other complex systems. The demonstration of drastically different behaviour from molecules based upon the system they are present in has implications for product development based upon tribological data in many industries, where by two seemingly similar systems which alone show closely matching friction profiles can show varied responses to the same formulation changes.

From the experiments conducted here it can be concluded that the post fermentation addition of proteinogenic amino acids could provide an effective methodology to reduce friction and potentially improve mouthfeel in both alcoholic and non-alcoholic commercial beer products. However, due to low solubility in this system only arginine is applicable, this finding could however be investigated in other beverage systems, where conditions are more conducive to dissolution of a wider range of amino acids. The improvement in lubricity seen here was found to be concentration dependent, indicating that at least 60 mM of arginine is required to produce any significant positive effect in either alcoholic or non-alcoholic beer systems. It was also demonstrated that in non-alcoholic beer addition of proteins could be used to improve upon predicted mouthfeel, although in this case the type of protein was crucial with lysozyme showing improvements and BSA degrading existing lubricity. Overall addition of post fermentation exogenous amino acids or protein represents a possible

avenue for the alteration of mouthfeel in beer products, with particular relevance to low/no-alcohol systems.

Conflicts of Interest

The authors declare no conflicts of interest relating to this work.

Funding Statement

Funding for this work was provided by The University of Birmingham Adrian Brown Scholarship in brewing research.

Acknowledgements

The authors would like to thank Michael-Alex Kamlow for assistance in the CAD for the tribology geometry and for assisting in reviewing this manuscript.

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Chapter 6- Conclusions and future work

6.1 Conclusions

The goal of this work was two-fold, to investigate the utility of tribology, later tribo-rheology in the assessment of mouthfeel properties of beer products and to elucidate which molecular components may be important or determinant in mouthfeel, particularly looking at low alcohol vs full strength beers. Initial work was carried out on a PCS Instruments Mini Traction Machine, but was quickly moved to a TA rheometer using a 3-ball on plate geometry design with a PDMS cast bottom surface.

The study aimed to establish a methodology which allowed for reproducible analysis of commercial beer products as well as investigate less complex single component and multi-component defined systems. In depth conclusions for each results chapter are included below.

6.1.1 Initial investigation of commercial beverages MTM

Initially no published work was available on instrumental assessment of beer mouthfeel, while many studies had made use of tasting panels the use of tribology or tribo-rheology was absent. Initially the commonly used dedicated tribometer Mini Traction Machine was used, this rolling ball style tribometer proved to be capable of measuring beer products but due to very high run-run variance it was challenging to produce data with acceptable error rates. It was noted that this setup was incapable of demonstrating a clear difference between water and several commercial beers indicating it was not sufficient for the aims of the study. This was likely caused by the settings required versus the normal values used on tribometers, the

normal force of 1N was the lowest selectable down force and showed high variance, possibly also linked to the secondary surface in use at this time (silicone elastomer). Run to run variance even using the same surface was found to be high and the compounded error rates and when new surfaces were utilized for other samples made the data unreliable for conclusions.

6.1.2 Initial investigation of commercial beverages Tribo-rheology

Following on from work conducted using MTM tribometers tribo-rheology was examined as an alternative, with PDMS disks. This produced significantly more reproducible data with improved run to run variance for both the instrument and disk-based errors. This methodology was found to be able to produce data of sufficient reproducibility to allow for T-Tests to be conducted with significant ($p=<0.05$) differences established even between the same breweries' full strength and alcohol-free versions of the same product. This was a key parameter to enable more detailed investigation, due to the focus of the work being around low alcohol beers. The method was also found to be effective at measuring pure water as a comparison point for both beers and other samples, from which some basic testing of other molecules could be conducted, ethanol, maltodextrin, maltose and sodium chloride all showed interesting behaviours indicting this was an avenue worth pursuing further.

6.1.3 Microstructure Tribo-rheology as an approach

Commonly tribology/tribo-rheology are performed on protype or completed products, analysing systems one molecule at a time to observe specific interactions is not common, this is largely due to the product focused nature of most tribology studies. Having

demonstrated the ability to show differences between low concentrations of the same inorganic salt, the basic components of a commercial beer were separated out, both organic and inorganic and tested individually with and without ethanol at concentrations previously measured in beer by other studies. These selected concentrations were not intended to mimic any specific beer or beer style but were simply the highest published concentration for that substance, this was done to allow for more individual molecules to be tested, rather than many concentrations. The results demonstrated a high level of unpredictability, but good reproducibility, with relatively low error rates compared to other published works. The finding that molecules below taste threshold at 14 parts per million were able to alter the behaviour of a far greater concentration of another molecule's behaviour allows for the idea to potentially manipulate specific molecules at levels undetectable to consumers, which then have an impact on the tribological behaviour of the product. It was also noted from this work that ethanol behaviour is not always positive for friction as is commonly thought, with some mixtures with ethanol showing markedly worse lubricity than without ethanol. This somewhat disbands any idea that low alcohol beer is simply missing ethanol and that is the only cause for any perceived defects, given the large effects other molecules demonstrated at concentrations hundreds of times lower than ethanol at 5%. In some cases even able to override the behaviour of ethanol and produce a novel lubricity.

Within this work several amino acids as well as a protein were examined, these produced unique results and suggested a possible avenue for further investigation, where due to rising popularity of protein based health drinks coinciding with low alcohol beer demand rising protein or amino acids could be used to improve the mouthfeel properties of beers. This is particularly relevant to low alcohol beers, as it is broadly illegal to advertise health benefits

from any alcoholic product so the addition of nitrogen sources to them is not desirable commercially.

6.1.4 Nitrogen Manipulation for lubricity

Following on from the findings of 6.1.3, it was decided that to allow more ready comparison a molar concentration would be selected for this study and given the exogenous nature of the amino acid/protein additions a value was selected at which all proteinogenic amino acids were soluble. This yielded demonstrates that lubrication properties even in purely aqueous systems when considering only a single class of molecule are still complex and challenging to predict. Significant correlations were not found between friction forces and polarity, size or acidity/alkalinity, indicating that either another single factor is determinant or that some combination of these and or other factors is used to determine lubricant behaviour. It was also concluded that the addition of post fermentation amino acids or proteins could produce a noticeable alteration in system lubricity while remaining soluble in commercial products, but the choice of amino acid was limited significantly by solubility in beer systems. It was also observed that low levels of some molecules could increase friction, as was previously observed in 6.1.2, suggesting a disruptive action on existing system lubrication.

6.2. Future work

6.2.1 Tribofilm Characterisation

An interesting avenue for future development on this project would include the characterisation of the observed effects in chemical/physical detail. This would involve the observation (electron microscopy) or characterisation (Fourier transform infrared spectroscopy) of surfaces in various states of the tribological process. For example, soaking of both PDMS and stainless-steel surfaces in mixtures known to cause specific responses could then be subject to scanning electron microscopy (SEM) to observe the deposition of material, primarily focusing on the observed pattern of atomic deposition to compare disruptive and additive interactions that were observed in figure 4.3. This physical characterisation could be repeated after subjecting the surfaces to tribological forces, determining if the observed increases in friction are related to film deformation or failure by direct observation. An alternative methodology would be to utilize Fourier Transform Infrared Spectroscopy (FTIR), which could be utilized to determine the chemical composition of the surface structure, this would likely prove more useful for mixtures where small molecules are concerned, as observation of inorganic deposition may be challenging with SEM. Again, the retesting of surfaces post tribology run could yield insight into the molecular detail of if and how films fail, as well as which surfaces are most important to the observed effects.

6.2.2 Correlation with human perception

An important but challenging future field would be the use of human tasters to observe the impact of the observed instrumental effects in human participants. This is a core issue with instrumental tribology, in that the measured effects may not be correlated well to effects noticeable or desirable to human tasters. A further challenge with this particularly, relating to this work is the unpalatable nature of many of the single molecules, with several of the molecules not being suitable for human consumption or at the least known to taste unpleasant alone. It has previously been observed that it is challenging for tasting panels to separate mouthfeel entirely from taste and could be expected to be especially challenging with unpleasant tastes showing minor differences in lubricity. Given the challenges associated with this approach focusing on the application rather than the pure science side would likely be more successful, investigating if the addition of soluble protein and or amino acids to beer products is noticeable or desirable for consumers. As the goal of many protein/supplement type beverages is frequently focused on functionality rather than purely quality, if the addition of amino acids or protein is possible without significantly altering consumer perception that could also represent an interesting avenue for investigation with obvious industrial application.

6.2.3 System replication for tribology

An unusual but possibly interesting avenue for further investigation would involve the chemical characterisation of a commercial product, measuring as many of the components as possible, detailing saccharides, inorganic ions, proteins, volatile organic molecules and others. Using this data a synthetic beer could be produced containing the same ingredients

as the commercial product, which can then be compared to the tribology from that original product. Having completed this, alternative versions could be produced, which lack individual components, this systematic approach could be used to identify which if any single molecules provide a major determinant factor of tribological behaviour. This method does face significant issues, especially with beer, due to the biological nature of beer it contains many different molecules, large numbers of which are difficult to purchase commercially as they are biological intermediates with no reasonable synthesis pathways for marketable quantities. Given this limitation, a less complete method could be attempted, whereby quantities of the original product are purified, e.g., a protein or saccharide fraction, which could then be used in a similar fashion as the totally synthetic approach to help identify molecules of interest in a fashion similar to the identification of the function of genes in an organism's genome. This separation would represent a significant challenge to achieve high enough purity, most likely requiring preparative HPLC or other liquid chromatography method.

Increased Complexity Modelling systems

While it was found that simple linear models with single variables were unable to describe the relationship between different compounds and friction, it is possible that a more complex predictive model could. This sort of model has been utilized previously but struggles with more complex mixtures, this challenge presents a possible application for artificial intelligence based learning models, where a model can be trained on existing samples to then predict the properties of others, being corrected and further honed with more information. This represents an interesting possible utilisation of the emerging technology but would require a significant body of data before beginning to generate useful results,

likely requiring a focus on single classes of molecule to build up its predictive power. A concern with this approach is the volume of data required may not be achievable in a realistic time scale, but this is yet to be examined practically.