

Assessing the factors affecting draught beer hygiene and validation of a novel dispense system

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Abstract

In 2018, a significant 2.2 billion litres of beer were sold on draught throughout the U.K. representing 41.8% of all beer sold. Assuring the quality of draught beer at the point of dispense should be of paramount importance to both brewers and retailers alike, improving profits and protecting brand reputation. However, they face many challenges in doing so, due to; variable line cleaning efficacy, product rate of sale and dispense system complexity.

This project set out to perform, for the first time, quantitative analysis of the U.K. beer on-trade marketplace with the primary focus on improving beer quality across the bar. Initially, the traditional beer forcing method was applied in a novel way, to measure beer quality directly at the point of dispense. Greater understanding of beer quality available to consumers was gained through a wide-ranging survey of beer quality in 65 on-trade accounts across the East Midlands was performed with extensive data analysis showing significant difference between beer styles, price doesn't always mean better quality and a higher number of taps on the bar negatively impacted beer quality.

Subsequently, a novel dispense system and four market leading line cleaning chemicals, were stringently tested for their hygienic performance using beer forcing, biofilm quantification and Headspace-Gas Chromatography to identify effective innovations in dispense line materials, cooling systems and cleaning methodology.

Finally, the project took a first look at the total microbiome of a beer dispense system, identifying microorganisms present in; the keg, biofilm and dispensed beer. Microorganisms such as *B. thurigiensis* and *R. mucilaginosa* were identified alongside the much-reported beer spoilers*; L. brevis, A. aceti* and *B. bruxellensis*

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List of abbreviations

AAB	Acetic acid bacteria
ABV	Alcohol by volume
BII	British Institute of Innkeeping
Bp	Base pairs
°C	Degree centigrade
FOB	Foam on beer
xg	Gravitational force
LAB	Lactic acid bacteria
w/v	Mass/volume concentration
MDP	Medium density polyethylene
µg/mL	Microgram/millilitre
µL	Microlitre
mg/L	Milligram/litre
mL	Millilitre
mV	Millivolt
nm	Nanometre
nM	Nanomoles
OD	Optical density
PCA	Plate count agar
QA	Quality assurance
QC	Quality control
RR	Raka-Ray agar
Rpm	Revolutions per minute
TCA	Tricarboxylic acid cycle
v/v	Volume/volume concentration
WLN	Wallerstein laboratory nutrient

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1 INTRODUCTION

1.1 Beer

The production and consumption of beer has occurred for millennia, evidence from a study in Israel suggests that fermented and alcoholic beverages were being produced 13,000 years ago. Whilst further archaeological evidence links alcohol production with the progression of humans from hunter gatherer communities to those of settled farmers and agriculture involving cereal domestication (Hornsey, 1999; Liu *et al.*, 2018).

Traditionally beer is produced from four main ingredients; Malt, typically malted barley however, many cereal crops have been utilized such as maize, oats, rice, rye, sorghum and wheat (Briggs *et al.*, 2004). Hops, a relatively late arrival on the brewing scene, first appearing in beer in A.D. 1079 (Bamforth, 2003), have antiseptic properties and allowed the preservation and bittering of beer. Water, referred to as 'liquor' accounts for 91-98% of the final beer (Briggs *et al.*, 2004) and its composition of salts render it suitable to brewing various styles. Locations such as Burton-upon-Trent and Dublin are famous for the quality of their liquor in producing pale ales and stouts respectively (Boulton and Quain, 2001; Briggs *et al.*, 2004). Finally, yeast, typically of the genus *Saccharomyces*, is the yeast that performs the fermentation of 'wort' using up the fermentable sugars and producing ethanol

(C_2H_6O) and carbon dioxide (CO_2) along with a variety of flavour compounds (Boulton and Quain, 2001).

Draught beer is the term given to beer that is typically dispensed from casks and kegs through beer lines and out of a tap directly into a glass for consumption. It has traditionally been the method of choice for beer provision in public houses and may also be used in restaurants, hotels and stadia.

1.1.1 Global beer market

Worldwide beer consumption is in a steady decline, 186.72 million kL of beer were consumed in 2017, a reduction of 0.2% year on year. This is mainly due to the decline in the traditional beer drinking markets of Europe and the flat North American market. Meanwhile in the same period, the emerging markets of Central and South America and Africa have grown by as much as 4.3%. China is the world's biggest beer market accounting for a fifth of global beer volumes (20.5%) (Yoshinori, 2019).

1.1.2 U.K. beer market

In the U.K. on-trade consumption of alcohol has declined by 38% since 2000, with beer's share of the market reducing from 69.1% to 53.7% (Sheen, 2018). Consumer behaviour has also changed in recent decades, in 1980, 78.8% of beer was served as draught beer yet by 2017 this has fallen to just 42.4%.

This reduction in draught beer volumes is due to several reasons. Notably, the U.K. on-trade has shrunk from 87.7% of all beer sales down to just 46.9% resulting in more beer being consumed outside of the traditional on-trade marketplace (Figure 1.1).

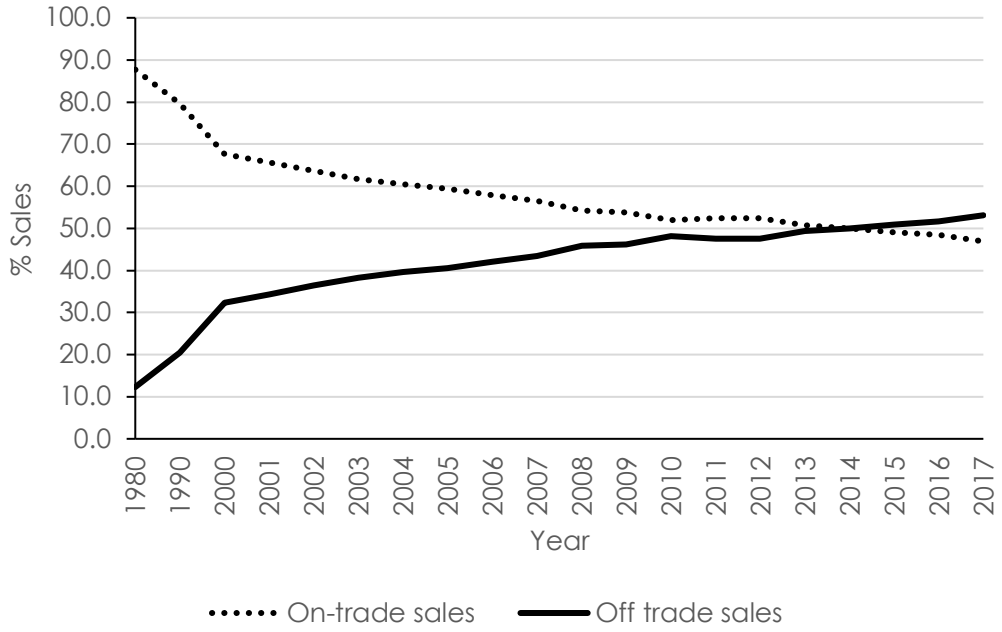


Figure 1.1: U.K. sales, on-trade vs off trade year on year. Data taken from the Statistical handbook - BBPA (Sheen, 2018).

Consumers are increasingly purchasing small pack products in bottle and can from supermarkets, as a cheaper alternative to draught beer. The growth and diversity of small pack beer being drunk at home has resulted in consumers moving away from drinking more expensive beer in public houses. (Sheen, 2018)

Public houses themselves have declined in number, the shrinkage of the on-trade market has coincided with the closure of 12,450 public houses across the United Kingdom since 2000. (Sheen, 2018)

Further reasons for the shift in drinking habits range from; beer duty, the smoking ban, responsible drinking initiatives and economic uncertainty (Quain, 2006).

1.2 The Brewing Process

1.2.1 Malting

Cereal grain (typically barley) is harvested, cleaned and stored prior to undergoing further processing via the 'malting process'. The goal of malting is to provide the nutrients and fermentable sugars for yeast growth and fermentation.

Malting starts with steeping, which leads to the initiation of germination. The barley grain is immersed in and/or sprayed with water (steep liquor), to raise the moisture content (the degree of steeping), to approximately 42-48% (Briggs, 1998; Kunze, 1999).

Steep liquor is changed at least once as it becomes contaminated with microorganisms (from the barley), microbial metabolites and dissolved substances. The 'green' malt is aerated regularly as CO_2 inhibits growth and because aerating malt reduces clumping (Briggs et al., 2004).

Germination initiates malt modification, there is root and leaf growth, grain modification and enzyme production. Hydrolases are released and partially breakdown the starchy endosperm releasing sugars and nutrients for yeast growth. Once the target

level of modification is reached the germination process is halted by kilning.

Kilning is principally used to stop further modification of barley malt and reduce the moisture content to a level below 6%. To achieve a range of malt profiles, the malt can be kilned for longer periods and at varying temperatures. For example, Pilsner/lager malt is kilned for a shorter period than the darker roasted malts used in dark beers (Briggs, 1998; Hornsey, 1999; Kunze, 1999).

1.2.2 Wort Production

1.2.2.1 Mashing

Malted barley grain which has been milled or supplied pre-crushed depending on the size and equipment availability of the brewhouse are mixed and in some cases supplemented with adjuncts. Adjuncts (wheat, maize etc) are cheaper sources of extract and in larger brewhouses can contribute to the grist bill. They may also provide useful characteristics, for example, wheat flour is often used to provide protein for good head formation and retention (Bamforth, 2003; Briggs *et al.*, 2004)

The mix of malt and adjuncts is known as the grist. The grist is hydrated as it passes into the mash tun, the water known as 'strike liquor' (Bamforth, 2003) is heated so that once all grist is in the mash tun the grain bed temperature is 66-67°C. This temperature is key as it enables efficient activity of the key enzymes; a-amylase and

β-amylase used in breaking down the starch into fermentable sugars. Depending on the mashing scheme various temperature rest periods can be employed to provide ideal conditions for different enzymes (proteinases, dextrinases, glucanases etc).

In most brewhouses on completion of mashing, the mash is transferred to another vessel or lauter tun. The lauter tun is designed to facilitate effective separation of the liquid 'wort' which contains the fermentable sugars and nutrients required by the yeast, from the grain. According to Darcy's equation (Bamforth, 2003)

$$\text{Rate of liquid flow} = \frac{\text{Pressure x Bed permeability x Filtration area}}{\text{Bed depth x Wort viscosity}}$$

Once run-off of wort from the lauter tun to the kettle or copper begins, sparging also starts. Hot liquor (76-78^{o}C) is sprayed over the grain bed raising the temperature to terminate any further enzyme activity and to recover any remaining sugars and nutrients still in the grain bed. The target gravity of the wort determines how much sparge liquor is used.

1.2.2.2 Boiling

Upon completion of wort transfer to the kettle/copper, the wort is boiled, and hops are added. The boiling of wort serves many purposes; to sterilise the wort (Storgårds, 2000; Bokulich and Bamforth, 2013; Paradh, 2015) and inactivate any malt enzymes

present, isomerisation of hop alpha acids providing bitterness (Sakamoto and Konings, 2003; Briggs *et al.*, 2004; Vaughan *et al*., 2005), aiding in wort clarification causing the formation of 'trub' which is the insoluble precipitate formed by polyphenols and proteins, and finally colour formation due to the formation of melanoidins from reducing sugars and amino acids at high temperatures (Boulton and Quain, 2001; Bamforth, 2003; Briggs *et al.*, 2004).

Boiled wort is cooled to < 20°C (depending on beer style) using a plate heat exchanger and aerated/oxygenated during transfer to the fermentation vessel.

1.2.3 Fermentation

Brewery fermentation is performed by yeast, typically *Saccharomyces cerevisiae* for ales, fermented at approximately 20°C and *Saccharomyces pastorianus* for lagers, typically fermented between 6 to 14 °C (Germany, Czech Republic) or 15-18 °C (UK). Some beer styles such as lambic and gueze (in Belgium) use either mixed or wild fermentations whereby diverse yeasts and bacteria are involved (Hornsey, 1999; Menz *et al.*, 2010; Suzuki, 2015).

Wort contains the required compounds for yeast growth namely, fermentable sugars, nitrogen source (amino acids, peptides) and

vitamins. In some ways, the production of beer is simply an outcome of yeast growth (Boulton and Quain, 2001).

The metabolic pathways the yeast uses to grow in fermentation are what differentiates brewing from yeast biomass formation. In wort, the high concentration of sugars combined with limited oxygen availability (at the beginning of fermentation only), trigger the yeasts to use glycolysis producing ethanol and CO_2 from the fermentable sugars. If the sugar concentration was low with oxygen present (continuously) then yeast growth would be oxidative using respiration with significantly more biomass formation and little alcohol production (Boulton and Quain, 2001; Bamforth, 2003).

In addition to ethanol production, the yeast forms aroma and flavour compounds during fermentation (Peddie, 1990; Verstrepen *et al.*, 2003; S. Saerens *et al.*, 2008). Yeasts are selected for fermentation based upon their ability to produce a desirable flavour profile combined with efficient ethanol production.

1.2.4 Conditioning and bright beer filtration

After primary fermentation is complete and prior to packaging the beer is termed 'green beer' (Krogerus and Gibson, 2013). This beer is transferred to a conditioning or maturation tank, where it is chilled (typically <1°C) and held for a short time (days) or, traditionally, many weeks. Although mainly about sedimentation

589 and removal of solids, residual yeast will utilise any fermentable
590 sugars and remove green beer off flavours. Further, additions
591 (sugar, colour, flavourings, bitterness etc) are made as this step.

592 Cold conditioning is used to allow any remaining yeast and 'debris'
593 to drop out of solution and sediment at the base of the vessel. The
594 reduced temperature enables the formation of protein polyphenol
595 complexes which sediment under gravity. This can be accelerated
596 with the addition of stabilizers such as Poly-vinyl-polypyrrolidone or
597 silica hydrogel. These compounds are removed during filtration
598 (Bamforth, 2003).

599 Undesirable flavour and aroma compounds that form such as
600 hydrogen sulphide and acetaldehyde can also be removed by
601 'gas washing' with CO_2 or N_2 during conditioning (Boulton and
602 Quain, 2001).

603 Bright beer filtration is traditionally performed with plate and frame
604 filters by a process known as depth filtration, the filter sheets are
605 made of cellulose and kieselguhr (or perlite). With pore sizes of 5 –
606 20 µm, this style of filter relies on the microorganisms being
607 mechanically trapped or adsorbed to the pores due to the
608 opposing charges present on the filter sheet (positive) and
609 bacterial cells (negative).

1.3 Packaging of beer

Finished beer is packaged into either small pack (bottle and can) or large pack (cask and keg). Keg beer is filtered and/or sterilised by sterile filtration or the pasteurisation process prior to keg filling. Cask beer is not filtered or pasteurised, and is a 'live' product (Pawlowsky, 1991; Livens and Pawlowsky, 2009; Nelson *et al.*, 2018) that requires the residual yeast to perform a secondary fermentation in the cask as part of the process.

Bottles and cans are filled and sterilised in a tunnel pasteuriser. Alternatively, the beer is flash pasteurised or sterile filtered before aseptic filling. In this case there is no further microbiological stabilisation process post filling and hence hygiene standards must be high to prevent a quality failure. As with cask beer, bottle conditioned beer (containing yeast) is not subject to microbiological stabilisation (Boulton and Quain, 2001).

1.3.1 Biological stabilisation methods

1.3.1.1 Pasteurisation

Traditionally beer has been microbiologically stabilized by using pasteurisation, a process that combines heat and time to kill microorganisms in the product. The beer must be subjected to sufficient 'pasteurisation units' (PUs) to render the product microbiologically stable. One PU is described as the 'biological

destruction obtained by holding a beer for one minute at 60 °C.' (Del Vecchio *et al*., 1951)

1.3.1.2 Tunnel Pasteurisation

Beer packaged in bottle and can are commonly packaged first and then pasteurised in batches in a tunnel pasteuriser (Briggs, 2004). In a tunnel pasteuriser each bottle or can is heated at the required pasteurisation temperature and held at the required time to ensure the sufficient killing of microorganisms. Typically, the temperature is 60 °C (140 °F) with a holding time of 20 mins. Strict control is applied to ensure the effective killing of microorganisms in the most energy efficient manner (Kunze, 1999; Briggs *et al.*, 2004)

A tunnel pasteuriser consists of zones, bottles move through these zones on a conveyor system, either a slat continuous conveyor or a walking beam conveyor. The walking beam is advantageous as the moving parts stay within one temperature zone. As the bottles move through the zones, they are heated by water jets, each zone increasing in temperature until the bottles reach the top temperature. The water is circulated through the pasteuriser and is recovered to cool the bottles once they have been pasteurized. This makes the process as efficient as possible (Kunze, 1999; Briggs *et al.*, 2004)

1.3.1.3 Flash Pasteurisation

Keg beer is treated prior to filling by flash pasteurisation. Beer in bulk is heated and cooled quickly by moving through a heat exchanger as it flows towards the keg racker.

There are four sections in a flash pasteuriser, firstly beer is pumped through to the regeneration section, at this stage beer is flowing counter-current to hot beer and is therefore pre-heated. The flow then carries on to the heating chamber where the temperature is raised to approximately 72°C, by passing counter-current hot water or steam. The beer is then held at this temperature for the desired time, normally this is in the range of 15-60 seconds. The hot beer flows back to the regeneration section to heat the incoming beer, the beer enters the cooling section where the beer flows counter current to glycol, brine or alcohol (Bamforth, 2003; Briggs *et al.*, 2004). The entire process from beer entering at 2-4°C, heated to approximately 72 °C and then cooled takes no longer than two minutes (Dymond, 1992). Due to its efficient design about 97% of energy used during flash pasteurisation can be recovered (Kunze, 1999). Flash pasteurisation has advantages over tunnel pasteurisation namely; smaller footprint, lower capital and operating costs and reduced exposure of beer to heat (preventing flavour deterioration) (Briggs *et al.*, 2004).

1.3.1.4 Sterile filtration

Sterile filtration is an alternative to heat treatment of beer. The major benefit is the negligible impact on beer flavour as there is no heating of the product. However, filling of final packages must be aseptic so contamination must be avoided downstream of the sterile filter (Kunze, 1999; Briggs *et al.*, 2004).

Membrane filters with good pressure stability are now widely used (Back, 1992; Asano *et al.*, 2007), capable of pore sizes of 0.2 – 0.45 µm, that achieve absolute sterility (Briggs *et al.*, 2004). They are however prone to blockages and several filters in succession of decreasing pore size are required to ensure a smooth operation (Kunze, 1999; Briggs *et al.*, 2004; Asano *et al.*, 2007).

1.3.2 Kegs and kegging

Kegs are commonly constructed of stainless steel, they are generally made in sizes of 50 and 100 Litres. However, increasingly, 30 L kegs are seen in the on-trade.

The keg itself has a single opening through which all the processes occur. The 'Barnes neck' contains the keg valve and the spear (a tube that runs from the valve to the base of the keg) through which all kegging processes are performed (Hornsey, 1999; Kunze, 1999; Briggs *et al.*, 2004).

Kegging is made up of a series of processes performed on an automated line. Returned kegs are drained and washed externally

before moving to the keg racker, consisting of successive heads for internal cleaning, sterilizing and subsequent filling with flash pasteurised bright beer (Hornsey, 1999; Kunze, 1999; Bamforth, 2003; Briggs *et al.*, 2004).

Because kegs are filled with pasteurised beer, they are 'commercially sterile' with no subsequent biological stabilisation process. The internal cleaning of kegs is performed with water at approximately 70 °C and caustic detergent. Following a series of water and detergent rinses, ensuring the entire internal surface is cleaned, the final rinse is purged with steam ready for sterilisation. During sterilisation the internal temperature of the keg reaches 105°C for a minimum of 30 seconds to 1 minute. Post sterilisation kegs are purged with CO_2 to drive out any remaining moisture, remove oxygen and create a back pressure so fobbing (excess foam generation) is reduced upon filling (Hornsey, 1999; Kunze, 1999; Bamforth, 2003; Briggs *et al.*, 2004).

Once filled and capped, kegs will have a lengthy shelf life of three months or more. Kegged beer dispense involves the attachment of a keg coupler (see 1.6.4), which is attached to the top of the spear and allows for the ingress of gas to drive beer out of the keg upon dispense. Kegs require no settling time this and the completely sealed construction makes them much simpler to handle than cask beer (1.3.3)

1.3.3 Casks and cask racking

Cask beer is a live product and does not undergo filtration or heat treatment. It relies on yeast to produce carbonation by secondary conditioning. Here, residual sugar or added priming sugar is fermented by yeast to produce CO_2 (Briggs *et al.*, 2004).

Casks are simpler than kegs, made of stainless steel, with two openings. One at the centre or 'belly' of the cask is the entry point of beer during the racking (filling) process. The second opening is a smaller one at the top of the cask which, prior to filling is sealed with a keystone.

Cask racking can be automated in larger breweries or performed manually in smaller breweries. Beer containing approximately 1-3x10^6 yeast cells/mL is racked into the cask with typically an addition of finings. Finings are used to facilitate the settlement of the yeast and other particulates prior to dispense of the beer. After the cask is filled, the opening is sealed with a plastic or wooden shive (Hornsey, 1999; Kunze, 1999; Briggs *et al.*, 2004).

Dispense of cask beer is practically more complex than keg beer. It is synonymous with the U.K. and is rarely found elsewhere in the world. Casks are placed on a stillage to settle. A tap is driven through the keystone and attached to the beer dispense line. The cask is also vented by the piercing of the shive with a peg known

as a spile. Cask beer can take 72 hours to settle and be ready for dispense.

A manual hand pump is used for the dispense of cask beer, as beer is pulled along the dispense line air is drawn into the cask through the hole in the shive. The ingress of air is a likely route for contamination with airborne beer spoilage organisms present in the cellar. This is one of the reasons cask beer once 'tapped and vented' should be on sale for a maximum of three days.

1.3.4 Small pack: Bottle and Can

The other major packaging option available to brewers is bottling and canning. Beer is treated similarly to that of kegged beer however, it is possible to pasteurise the beer after filling in these smaller packaging types. Bottle and can sales are predominantly through the off-trade such as in supermarkets but are also readily available in many on-trade accounts such as, pubs, restaurants and hotels.

1.4 Draught beer quality

1.4.1 Definitions of quality

- 'A beer that is responsibly produced, using wholesome ingredients, consistent brewing techniques and good manufacturing practice, which exhibits flavour characteristics that are consistently aligned with both the

brewer's and beer drinker's expectations' – Brewers Association

- 'Quality is the achievement of consistency and the elimination of unwanted surprises' (Bamforth, 2016)

Consistency of product is a mark of quality, established large multi-national and regional brewers can achieve control over the process that ensures a consistent product. However, once the beer is released from the brewery this control is lost and it is dependent on the good practices of distributors and retailers.

1.4.2 Parameters of draught beer quality

Some of the key parameters of beer quality are pH, flavour profile, colour, bitterness, ABV, dissolved O_2 and CO_2. These are the parameters measured by a brewery quality assurance (QA) or quality control (QC) laboratory. If the final product fails key quality limits, then it will be rejected and not go to trade.

Quality will be expressed in different ways to brewers by consumers. The consumer will initially judge a beer with their eyes, nose and throat (Bamforth, 1998), they will notice obvious flaws such as haziness, poor serving temperature and may even detect off flavours, such as the 'vinegary' (acetic acid) or 'buttery' (diacetyl) flavours produced by contaminants of draught beer.

1.4.3 Draught beer quality

The only hygiene standard regarding draught beverages is the German DIN standard (Deutsch Institut fur Normung). DIN 6650 and its component parts cover areas such as hygienic design and materials used. 6650-6 provides guidelines for categorising the hygiene of draught beverage lines based on spread plates. There is no recommendation of media and all draught beverages be it wine, beer or water are treated the same.

The guidelines in the DIN standard are: 1000 cfu/mL is acceptable, 10,000 cfu/mL suggests cleaning is necessary and a count of 50,000+ cfu/mL is unacceptable.

1.5 Microbiology of beer

Beer is considered a safe beverage consisting of; ethanol often around 4-5% but up to as high as 10% or more, a low pH around 4.1 providing an acidic environment restricting the ability of many bacteria to grow and a low level of nutrient sources. The increasingly reduced levels of dissolved oxygen in keg beer provide an anaerobic medium, which consequently limits the growth of aerobic microorganisms (Vaughan *et al*., 2005; Suzuki *et al.*, 2006; Menz *et al.*, 2010; Vriesekoop *et al.*, 2012; Suzuki, 2015). However, certain species of bacteria have found it possible to not only survive but grow in beer at various stages of processing. This

growth is synonymous with spoilage of the final product due to haze formation and altered aroma and flavour profiles.

In a brewery any organism that is not deliberately introduced and yet is able to survive and proliferate in the environment may be known as a spoilage organism (Jespersen and Jakobsen, 1996). Typical beer spoilage organisms include both Gram-positive organisms such as lactic acid bacteria and Gram-negative organisms such as acetic acid bacteria. Yeast is also a major player in beer spoilage and any yeast present other than that of the desired brewing strain is termed a 'wild yeast' (Wiles, 1953; Hemmons, 1954, 1955; Richards, 1968; Campbell and Msongo, 1991).

Processes within the brewery are strictly controlled and keg beer will normally leave the brewery, in a 'commercially sterile' state containing very low levels of yeasts and bacteria (<1cfu/litre, (Boulton and Quain, 2001). It is essential that cellars and dispense systems employ a consistent, effective cleaning and hygiene regime to maintain product quality.

1.5.1 Gram-positive beer spoilers

Of the Gram-positive beer spoilage bacteria, members of the lactic acid bacteria (LAB) are reported to be the most culpable of causing spoilage incidents in breweries (Back, 1994; Hutzler *et al.*, 2012; Koob *et al.*, 2014). These incidents were predominantly

caused by either *Lactobacillus or Pediococcus and less so by; Lactococcus, Leuconostoc, Streptococcus Enterococcus*, all of which are hop sensitive and unable to grow in beer (Suzuki, 2015). Outside of the LAB there are few Gram-positive organisms reported to be capable of beer spoilage.

1.5.1.1 Lactobacillus

The *Lactobacillus* genus is the largest within the LAB and consists of many species (Sakamoto and Konings, 2003). They are Gram-positive, rod-shaped organisms that are non-motile. They can tolerate oxygen and some species are 'capnophilic' meaning the presence of carbon dioxide encourages their growth. Furthermore, they are able to tolerate low pH and some species are resistant to hops (Sakamoto and Konings, 2003; Suzuki, Iijima, Sakamoto, *et al.*, 2006; Bokulich and Bamforth, 2013; Boulton, 2013). *Lactobacillus* can be classed as either homofermentative or heterofermentative depending on the metabolic pathways used. Homofermentative species produce almost solely lactic acid whereas heterofermentative species produce a mixture of lactic acid, CO_2, acetic acid and/or ethanol (Priest, 1996).

Lactobacillus species are commonly used in the processing of many fermented food products. Certain beer styles such as Lambic, Gueze and Kettle Sour beers, are fermented using a culture of lactic acid bacteria added during the fermentation

stage to produce an apparent sourness due to acid production. Further products such as wine and yoghurt can also be produced with LAB involvement (Sakamoto and Konings, 2003).

Despite the pleth*ora* of *Lactobacillus* species identified, not all of them are capable of growth in beer and hence most major quality issues are caused by a small number of species within the genus. According to the literature the most hazardous species is *Lactobacillus brevis*, and others shown in Table 1.1 (Suzuki, 2015).

Table 1.1 Beer spoilage organisms and % incidents caused.

Genus/species	1980-1990 (%)	1997-2002 (%)	2010-2013 (%)
L. brevis	40	45	47.8
L. lindneri	25	8	4.9
Other *Lactobacillus* spp.	6	12	25.9
Ped. Damnosus	17	16	6.3
Other *Pediococcus* spp.	N/A	N/A	0.7
Pectinatus spp.	4	6	3.4
Megasphaera cerevisiae	2	3	2.0
Yeasts	N/A	10	N/A

Data collated from (Back, 1994, 2003, 2005; Hutzler et al., 2012; Koob et al., 2014; Suzuki, 2015)

Due to its predominance in beer and brewing *L. brevis* is widely studied, it is obligately heterofermentative (Priest, 1996), grows

optimally at 30°C and at a pH of 4-6. It has also shown resistance to hop compounds (Sakamoto and Konings, 2003). Other commonly encountered species are *L. lindneri,* a highly hop resistant species which is also the most tolerant of thermal treatments in the genus. *L. casei, L. plantarum, L. curvatus* and *L. buchneri* are also regularly reported offenders. (Sakamoto and Konings, 2003; Boulton, 2013; Suzuki, 2015).

Spoilage by *lactobacillus* causes several effects on the final product, ranging from a 'silky' turbidity and rope formation, through to souring by acidification (Ingledew, 1979; Jespersen and Jakobsen, 1996; Bokulich and Bamforth, 2013). Alterations to beer flavour most notably the production of a 'buttery' taste is due to diacetyl. Although this is formed and removed by yeast during fermentation, it has a low flavour threshold and can be produced in high quantities by contaminating LAB (Jespersen and Jakobsen, 1996; Priest, 1996; Boulton, 2013).

1.5.1.2 Pediococcus

The second main genus of beer spoilers in the LAB group is *Pediococcus*. These organisms are Gram-positive, non-motile (Boulton, 2013) and as the name suggests cocci in shape. They grow optimally at 21-25°C, however, are capable of growth at 9-12 °C and a pH range of 3.5-5.5. They occur in pairs and/or tetrads

(Ingledew, 1979; Jespersen and Jakobsen, 1996; Sakamoto and Konings, 2003), because they divide in two planes (Priest, 1996). There are fewer species within this genus than that of the *Lactobacillus*. Genetically the two genera are related and *Pediococcus* fall in the same cluster as the beer spoilers *L. casei* and *L. brevis*. The most problematic of the genus is the species *P. damnosus (*see Table 1.1) but more species have been isolated from breweries; *P. inopinatus, P. claussenii P. dextrinicus* (Dobson *et al.*, 2002; Back, 2005; Iijima *et al.*, 2007).

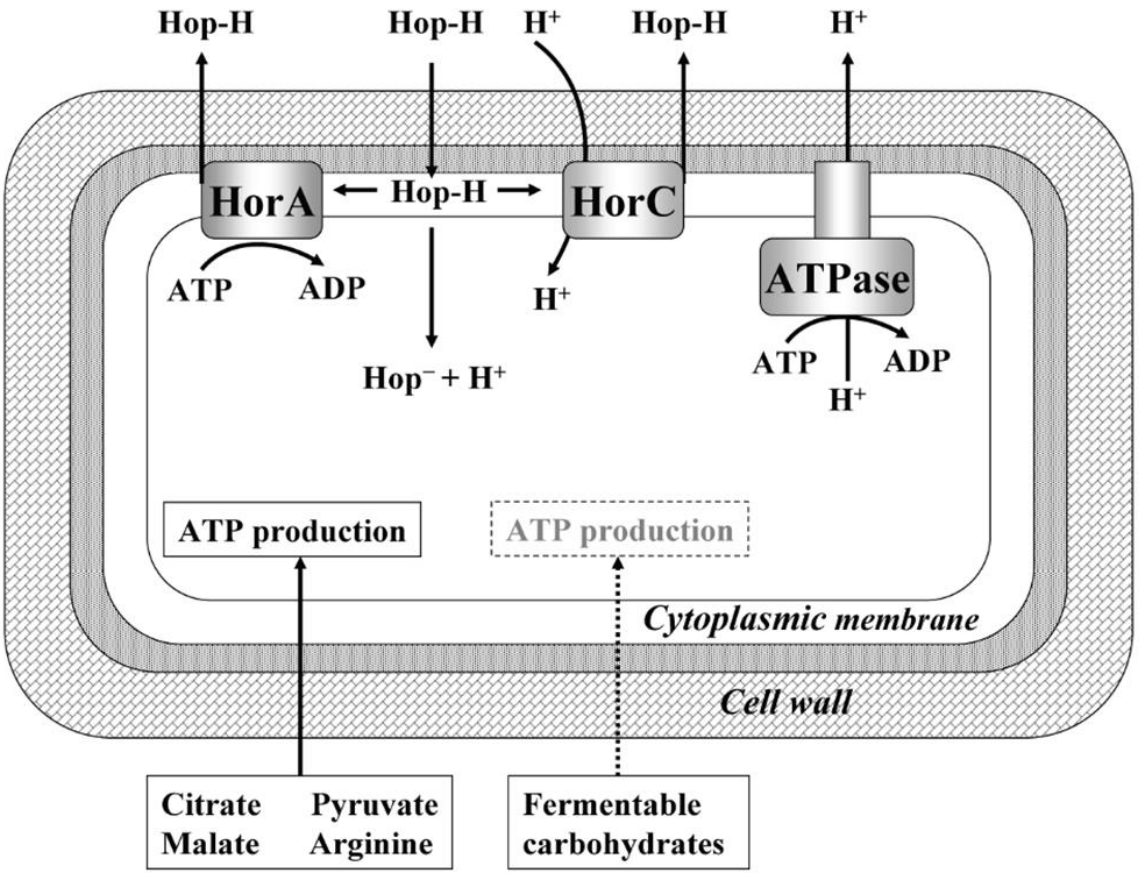
Pediococcus species are strictly homofermentative and produce lactic acid from the fermentation pathway. They are also produce excessive amounts of diacetyl from pyruvate (Ingledew, 1979). Furthermore, they produce ropiness and turbidity in beer caused by the production of polysaccharide capsules (Ingledew, 1979; Simpson and Taguchi, 1995; Jespersen and Jakobsen, 1996; Bokulich and Bamforth, 2013).

1.5.1.3 Hop Resistance in LAB

Hops are added to provide bitterness in beer but also show antibacterial activity. Here, the hop bitter acids such as, cis and trans-isohumulone, act as ionophores, trapping protons and exchanging them for divalent metal ions across the membrane of LAB (Simpson, 1993; Simpson and Taguchi, 1995).

Despite this antibacterial activity, some strains of *Lactobacillus* and *Pediococcus* are hop resistant, which is strain and not species dependent (Rinck and Wackerbauer, 1987; Simpson and Taguchi, 1995; Storgards *et al.*, 1998; Suzuki *et al.*, 2002; Suzuki *et al.,* 2004a) This makes it difficult to identify hop resistant strains by conventional species specific microbiological techniques such as colony morphology, catalase testing and other biochemical tests (Fernandez and Simpson, 1993; Suzuki, *et al.,* 2004a, 2004b).

Two genes have been found to confer resistance to hop compounds, *HorA* and *HorC*. *HorA* is an ATP dependent multi drug transporter, whilst *HorC* is a proton motive force dependent multi drug transporter (Sakamoto *et al.*, 2001; Iijima *et al.*, 2005, 2006). As undissociated hop compounds enter the cell membrane they are pumped out by one of the two transporters. However, not all will be removed, and some will enter the cytoplasm and dissociate. This would cause a change in internal pH, leading to cell death but, this is counteracted by a third mechanism, a proton translocating ATPase that excretes protons across the cell membrane thus maintaining internal pH (Sakamoto *et al.*, 2002). For these resistance systems to function, energy is generated from the metabolism of organic acids and on occasion, fermentable sugars (Suzuki *et al.*, 2005).



936

937 **Figure 1.2:** The proposed hop resistance systems in hop resistant beer
938 spoilage LAB. Two multi drug transporters, *HorA* – ATP dependent
939 and *HorC* – PMF dependent. A third mechanism to maintain internal
940 cellular pH by way of a proton translocating ATPase is also shown.

941 ***1.5.1.4 Other Gram-positive beer spoilers***

942 Aside from the LAB group, other recognised Gram-positive
943 bacteria that are associated with breweries and potential beer
944 spoilage consist of the spore forming bacteria; *Bacillus, Clostridium*
945 and *Paenibacillus* (Takeuchi *et al.*, 2005; Haakensen and Ziola,
946 2008). These organisms are generally sensitive to low pH and hop
947 compounds (Priest, 1996). However, some strains of both *Bacillus*
948 *cereus, Licheniformis* and *Paenibacillus humicus* have been found

to have the hop resistance gene *HorA* (Haakensen and Ziola, 2008).

One other organism of note is *Kocuria kristinae* previously referred to as *Micrococcus kristinae* (Bokulich and Bamforth, 2013). *Kocuria kristinae* has been found to be hop resistant, acid tolerant and compared to other strictly aerobic *Kocuria* species, it is facultatively anaerobic. The growth of *Kocuria kristinae* is promoted by oxygen and so any beer with elevated dissolved O_2 levels would be at risk especially if coupled with low bitterness (Suzuki, 2015). Spoilage by this organism yields a 'fruity aroma and an atypical taste in beer' (Back, 1981).

1.5.2 Gram-negative beer spoilers

Gram-negative bacteria associated with beer spoilage and brewery contamination are the acetic acid bacteria (AAB), *Acetobacter and Gluconobacter*, together with the *Enterobacteriaceae, Zymomonas, Megasphaera and Pectinatus.* The AAB are commonly associated with draught beer spoilage, (notably cask beer) and are; ubiquitous in the environment, hop resistant and capable of growth at pH of 3-4 (Holt *et al.*, 1994; Sakamoto and Konings, 2003; Paradh, 2015).

1.5.2.1 Acetic acid bacteria (AAB)

As many as 12 genera exist in the AAB (Sengun and Karabiyikli, 2011) however, there are two main genera which are the focus of

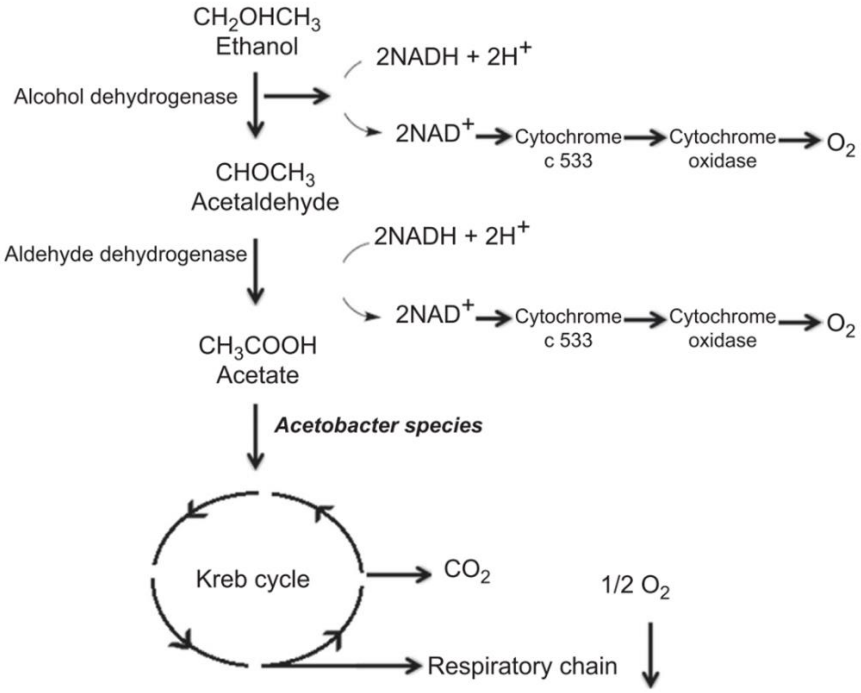
beer spoilage research, *Acetobacter and Gluconobacter*. Within these genera three main species are associated with beer spoilage: *Acetobacter aceti, Acetobacter pasteurianus* and *Gluconobacter oxydans*.

The AAB are Gram-negative or Gram-variable, non-spore forming short rods that occur singly, in pairs or in chains growing optimally at a pH of 5-6.6 yet capable of growth at acidic levels of pH 3-4 (Holt *et al.*, 1994; Paradh, 2015). They are motile, with acetobacter having petrichiously arranged flagella whereas gluconobacter have a polar arrangement (González *et al.*, 2005). They can survive high ethanol levels of >10% and are resistant to hop bitter acids (Ingledew, 1979; Vriesekoop *et al.*, 2012).

The AAB are considered strict aerobes yet, some isolates from draught beer have been found to be microaerotolerant (Van Vuuren and Priest, 2003) and ubiquitous in the environment. They were previously of great concern as beer spoilers in the brewery however, improvement in understanding, equipment and technology has resulted in minimal impacts on the brewing process and packaged beer. However, they are commonly found to be contaminants of draught beer systems, particularly cask beer where air is drawn into the cask to displace beer during beer dispense (Ingledew, 1979; Jespersen and Jakobsen, 1996;

994 Sakamoto and Konings, 2003; Roberts *et al.*, 2006; Vriesekoop *et*
995 *al.*, 2012; Bokulich and Bamforth, 2013).

996 A contamination of AAB will lead to the production of acetic acid
997 and haze caused by the formation of a pellicle on the surface of
998 the beer. They form acetic acid by the oxidation of the ethanol
999 (Figure 1.3) to acetaldehyde and then to acetic acid via alcohol
1000 dehydrogenase and aldehyde dehydrogenase. Acetobacter are
1001 also capable of further oxidising organic acids to CO_2 and H_2O
1002 using the Krebs/TCA cycle (Ingledew, 1979; Jespersen and
1003 Jakobsen, 1996; Saeki *et al.*, 1997; Paradh, 2015).



1004

1005 **Figure 1.3:** Oxidation of ethanol to acetaldehyde and acetic acid,
1006 catalysed by alcohol dehydrogenase and acetaldehyde dehydrogenase.
1007 *Acetobacter* only are capable of further oxidation of organic acids.
1008 (Paradh, 2015).

Effective cleaning and hygienic practices coupled with minimizing oxygen ingress into the beer are the best way to manage AAB contamination.

1.5.2.2 Megasphaera

This genus currently has three recognised beer spoilage species; *Megasphaera cerevisiae, Megapshaera paucivorans* and *Megasphaera sueciensis.* They are non-motile, non-spore forming cocci that are strictly anaerobic (Juvonen and Suihko, 2006; Juvonen, 2015), which grow at 15 – 37°C and a pH ≥4.1(Haikara, 1989).

Whilst better oxygen control supressed AAB contaminations in breweries, it provided a new niche for strictly anaerobic organisms such as *Megasphaera and Pectinatus* (see 1.5.2.3) (Sakamoto and Konings, 2003; Vaughan, O'Sullivan and Van Sinderen, 2005). Incidents of *Megapshaera* contamination are associated with unpasteurised beer or low alcohol beverages (Vaughan et al, 2005; Suzuki, 2011; Juvonen, 2015). These bacteria have been recovered from brewery filling halls on equipment such as fillers and conveyors but also from the environment in water pipes and condensation droplets (Paradh *et al.,* 2011).

Beer spoilage caused by *Megasphaera* results in turbidity, butyric acid, hydrogen sulphide (H_2S) production together with a faecal odour. They have a fermentative metabolism and utilise organic

acids such as pyruvic and lactic acid as their carbon sources. (Satokari *et al.*, 1998; Sakamoto and Konings, 2003; Juvonen and Suihko, 2006; Juvonen, 2015).

1.5.2.3 Pectinatus

Lee et al. (1978) first described the genus *Pectinatus* after isolating it in a brewery. Spoilage Incidents with this organism appeared at a similar time in Germany and Scandinavia (Haikara *et al.*, 1981). There are three recognised species, *P. cerevisiiphilus, P. frisingensis* and *P. haikarae*. They are Gram-negative, non-spore forming curved helical rods occurring singly, in pairs or rarely in chains. They are motile with a distinct flagella pattern in which they are formed only on one side of the cell and they lose motility as they age (Haikara *et al.*, 1981; Schleifer *et al.*, 1990; Suzuki, 2011; Juvonen, 2015).

Pectinatus species are most commonly isolated from brewery filling hall environments, as these tend to be humid and nutrient rich due to the presence of spilt beer. They have also been found in fermentations and are capable of inhibiting growth and ethanol production of *Saccharomyces cerevisiae* (Lee *et al.*, 1980; Chowdhury *et al.*, 1997; Haikara and Helander, 2006; Suzuki, 2011).

Like *Megasphaera, Pectinatus* is an obligate anaerobe, hop tolerant (Back, 2005), capable of growth at higher ethanol levels (3.7-4.4% abv) but not above 5.2% (Haikara *et al.*, 1981; Haikara

and Helander, 2006), it grows optimally at 30-32°C but is capable of surviving at temperatures between 8-15°C (Chowdhury, Watier and Hornez, 1995), a pH of ≤4.1 inhibits the growth of *Pectinatus* species, although their tolerance to acidity is affected by the levels of ethanol present in the beer. The most important barrier to spoilage by *Pectinatus* is the presence of oxygen, it is suggested that dissolved oxygen levels higher than 0.3 mg/L inhibit growth of both *Megasphaera* and *Pectinatus* species. However, *Pectinatus* has been shown to grow in wort containing as high as 0.96 mg/L of oxygen (Chowdhury et al., 1995).

Contamination with *Pectinatus* species leads to excessive turbidity and hydrogen sulphide (H_2S) giving a strong "rotten egg" aroma. Organic acids such as propionic, acetic and succinic acids can be produced as the end products of fermentative metabolism. Of note is the ability for *Pectinatus* to metabolize lactic acid, which suggests the potential for growth promotion where LAB contamination exists (Juvonen, 2015).

1.5.2.4 Other Gram-negative beer spoilers

There are two other recognised Gram-negative, anaerobic beer spoilers, the genera; *Selenomonas* namely *Selenomonas lactifex* and *Zymophilus*, the latter of which is phylogenetically similar to *Pectinatus* (Jespersen and Jakobsen, 1996). Their capacity for beer spoilage has received little attention as the primary focus has been

on the aforementioned organisms. *Selenomonas lactifex* is capable of producing acetic, propionic and lactic acids from glucose unlike most other species of *Selenomonas* (Schleifer *et al.*, 1990). *Zymophilus* species show similar beer spoilage to that of *Pectinatus* species, notably production of acetic, lactic and propionic acids (Schleifer *et al.*, 1990; Juvonen, 2015; Paradh, 2015).

Zymomonas mobilis has been isolated from primed beer, where sugar has been added to the cask for secondary conditioning. It has never been isolated from lager beer (Sakamoto and Konings, 2003). It is an aerotolerant anaerobe, cocci in morphology which grows above pH 3.4 and below 10% abv. It can utilize a narrow spectrum of sugars; glucose, fructose and sucrose, and spoilage with this organism leads to production of H_2S and acetaldehyde, whilst a heavy turbidity is also caused (Ingledew, 1979; Jespersen and Jakobsen, 1996; Sakamoto and Konings, 2003).

Beer associated *Enterobacteriaceae; Citrobacter, Enterobacter, Klebsiella, Rahnella* and *Hafnia* are more commonly associated with spoilage of wort and the production of off flavours that remain in the finished beer such as dimethyl sulphide (DMS), higher alcohols and phenolic compounds (Ingledew, 1979; Jespersen and Jakobsen, 1996; Fielding et al., 2007; Paradh, 2015).

Members of the *Enterobacteriaceae* are frequently associated with brewery biofilms and their production of exo-polysaccharides (EPS) protects the biofilm (Sutherland, 2001) from cleaning and sanitisation. They have been isolated from biofilms found in brewery bottling plants and beer dispense lines (Casson, 1982; Storgårds *et al.*, 2006; Storgårds and Priha, 2009; Priha *et al.*, 2015).

1.5.3 Yeasts

As well as the recognised bacterial beer spoilers, beer is subject to spoilage by diverse yeasts. They are classified into two main groups: *Saccharomyces* and *non-Saccharomyces*. Any yeast found to be present in a beer that is not the production yeast strain is a 'wild yeast'. Like bacteria, the growth and presence of these wild yeasts causes changes to aroma, flavour and clarity.

Yeast genera that have been associated with beer spoilage incidents include; *Saccharomyces, Brettanomyces, Pichia, Kloeckera, Debaromyces, Hansenula, Torulopsis, Rhodotorula* and *Candida* (Wiles, 1953; Hemmons, 1954; Hough *et al.*, 1976; Harper, Hough and Young, 1980; Casson, 1985; Quain, 2015).

1.5.3.1 Saccharomyces

Saccharomyces yeasts are the archetypal brewing yeasts - *S. cerevisiae* is used to produce ales and *S. pastorianus* for lager type beers (Bokulich, Bamforth and Mills, 2012).

Contamination with wild *Saccharomyces* yeasts causes the production of phenolic off-flavours and super attenuation (Jespersen and Jakobsen, 1996; Bokulich *et al*., 2012). The production of phenolic flavours is due to the decarboxylation of phenolic acids. Whilst super attenuation is due to extracellular glucoamylases breaking residual dextrins to sugars for further fermentation. (Jespersen and Jakobsen, 1996).

1.5.3.2 Non-Saccharomyces

Many genera of yeasts have been encountered in surveys of microorganisms in draught beer. *Brettanomyces* are the most commonly found non-*Saccharomyces* yeasts and contribute to the fermentation of beer styles such as lambic and gueze together with 'wild' or spontaneous fermentations for craft beers. *Brettanomyces* species produce a range of aroma compounds such as caprylic, capric fatty acids and their ethyl esters, which when produced in excessive amounts can convey, solvent-like, buttery and medicinal off flavour/aroma (Quain, 2015; Capece *et al.*, 2018).

Pichia, in particular *P. membranifaciens* has been regularly found in draught (predominantly cask ale) beers (Wiles, 1953; Hemmons, 1954, 1955; Hough *et al.*, 1976; Harper et al, 1980; Quain, 2015) whilst other species of *Pichia* have been associated with brewery tanks and biofilms in packaging plants (Storgårds *et al.*, 2006; Timke

et al., 2008; Pham *et al.*, 2011). *Pichia* yeasts are non-fermentative (Pham *et al.*, 2011), capable of surviving at low pH and can grow, albeit at a reduced rate, under anaerobic conditions. When in a reduced oxygen atmosphere *P. anomala* was found to produce increased levels of ethanol and glycerol (Passoth *et al.*, 2006), whilst spoilage characteristics associated with *Pichia* include, turbidity and ester off flavour production (Pham *et al.*, 2011).

Other yeasts genera associated with draught beer are: *Candida, Torulopsis Kloeckera* and *Rhodotorula.* All of these yeasts are capable of affecting the aroma and flavour profiles of the beers in which they are present (Quain, 2015). *Rhodotorula* is also a recognised early biofilm coloniser (Riedl *et al.*, 2019)

1.5.4 Biofilms

Microorganisms are ubiquitous in the environment. They are capable of establishing complex communities through their association to surfaces and subsequent production of biopolymers (mainly exopolysaccharides) that give structure, protection and allow for the sharing of nutrients (Storgårds *et al.*, 2006; Bridier *et al.*, 2015; Quain, 2015; Merino *et al.*, 2019).

Development of a biofilm consists of five stages (Figure 1.4); initial reversible attachment, irreversible attachment, colonisation and initial production of polymer matrix, maturation and subsequent proliferation by the release of cells to colonise nearby areas

(Zottola and Sasahara, 1994; Quain and Storgards, 2009; Quain, 2015).

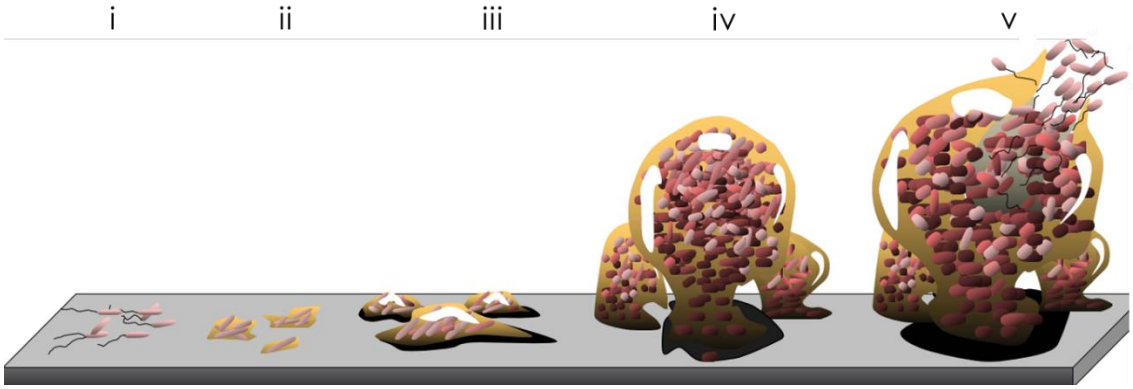


Figure 1.4: The five stages of biofilm formation; i) initial reversible attachment, ii) irreversible attachment, iii) colonisation, iv) maturation and v) proliferation (Monroe, 2007).

Biofilms are associated with causing infections in clinical settings and harbouring pathogens in food and beverage production sites (Bridier *et al.*, 2015). Food borne pathogens such as *Bacillus, Salmonella* and *Escherichia coli* have been identified in biofilms in food production sites such as dairies, poultry farms and egg processing plants. They are capable of forming on many surface materials such as nylon, stainless steel, plastic and glass (Bridier *et al.*, 2015; Merino *et al.*, 2019) all of which are used in beer dispense systems.

The sessile (attached) cells present in mature biofilms show increased resistance to antibiotics, preservatives, cleaning chemicals and mechanical action compared to their planktonic

(floating) counterparts. They become more resilient due to a combination of innate and induced factors (Anderson and O'Toole, 2008). These factors include protection from biocides afforded by the extracellular matrix, preservation of atmospheric conditions and the ability to share nutrients produced by the various metabolic systems present in the community by movement through the liquid matrix (Figure 1.5) (Davey and O'Toole, 2000; Anderson and O'Toole, 2008).

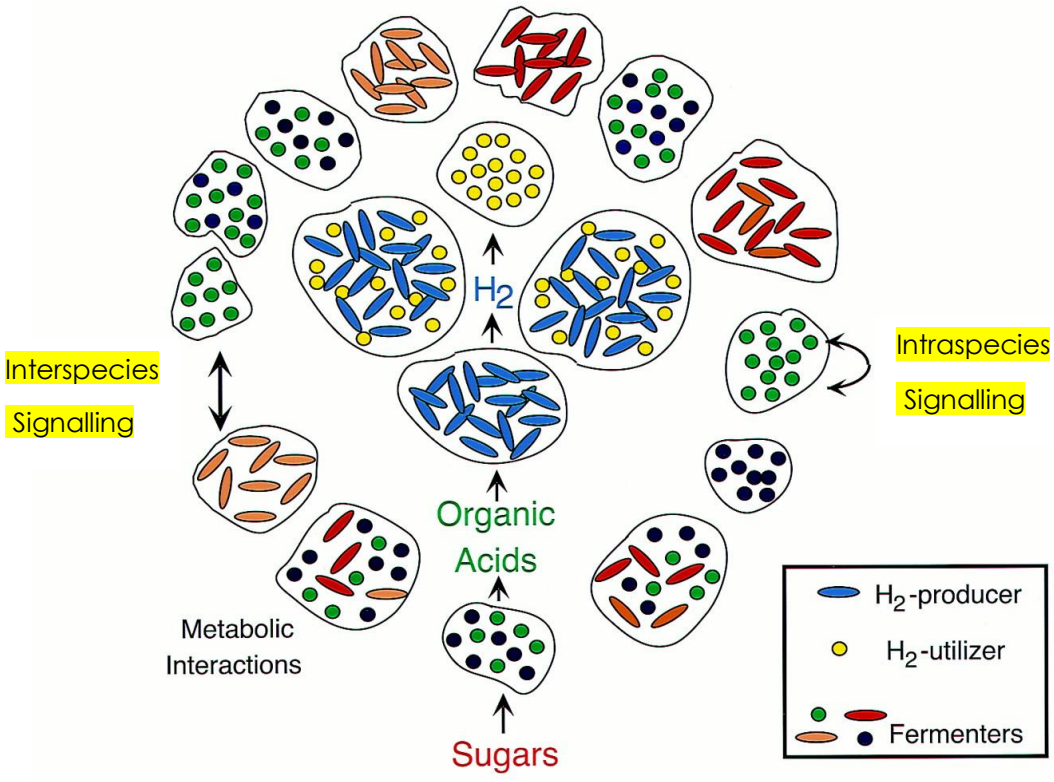


Figure 1.5: A simplified view of complex biofilm community highlighting the pathways utilised by constituent microorganisms to communicate and share nutrient molecules (Davey and O'Toole, 2000).

1.5.4.1 Biofilms in brewing

Within brewing, biofilms present in the brewery have been studied for their methods of attachment, resilience to cleaning and for the evolution of their constituent microorganisms (Storgårds *et al.*, 2006; Timke *et al.*, 2008; Storgårds and Priha, 2009).

There has been much less attention afforded to the presence of biofilms in beer dispense lines (Quain, 2015). It is accepted that they are present in beer dispense systems that are poorly maintained and have undergone infrequent or improper line cleaning (Storgårds, 2000; Walker *et al.*, 2007).

Aerobic or microaerophilic, Gram-negative bacteria such as the *Acetobacter* and *Gluconobacter* are commonly found to be early initiators of biofilm formation along with other Gram-negative non-beer spoilage bacteria (Quain and Storgards, 2009; Riedl *et al.*, 2019). Biofilm complexity increases with the association of yeasts, mainly wild non-*Saccharomyces* (Timke *et al.*, 2005) with *Rhodotorula* being commonly found as an early coloniser of brewery plant (Riedl *et al.*, 2019), followed by colonisation with *Saccharomyces* yeasts and beer-spoilage Gram-positive bacteria such as *Lactobacillus*. Once associated, yeasts are capable of producing pseudohyphae during biofilm maturation creating stronger attachment and spreading of the biofilm (Riedl *et al.*, 2019)

The structure of biofilm in a beer dispense line has not been studied though as we know that, the type of flow in a beer dispense line is laminar. Biofilms formed in this style of flow have previously been identified as patchy and formed of rough round cell aggregates (Davey and O'Toole, 2000).

The best protection from biofilm formation is regular and effective line cleaning. Cleaning is not 100% effective and there is a residual level of microorganisms present in the beer dispense system post cleaning, without regular cleaning these microorganisms will be able to re-form and re-colonise the beer line quickly (Storgårds, 2000; Quain and Storgards, 2009; Quain, 2015).

1.6 Flavour compounds produced by microbial spoilage

As a result of microbial growth and metabolism, sensorial changes to the beer occur. Both flavour and aroma are affected by the development of compounds, regarded as off-flavours in beer. These compounds alter the desired flavour profile of the product, causing negative consumer experiences.

The most commonly produced compounds include organic acids, higher alcohols/fusels, phenols and vicinal diketones.

Acetic and lactic acid are the two main organic acids produced, acetic acid is formed by the oxidation of the ethanol present in beer, as part of this oxidation, acetaldehyde is also formed (Figure

1.3), acetic acid is produced by both acetic acid bacteria and heterofermentative species of *lactobacillus*.

Homofermentative species of *lactobacillus* and *pedicoccus*, produce lactic acid by glycolysis, utilising residual hexoses present in beer. Heterofermentative lactobacillus also use this pathway, but also have the ability to utilise pentoses as they have the enzyme phosphoketolase present (Vandenberghe *et al.*, 2018).

Vicinal diketones (VDKs) impart a buttery, butterscotch flavour to beer and are a metabolic product of yeast fermentation, 2-3-butanedione known as 'Diacetyl' is the most problematic of these compounds due to its low flavour threshold. VDKs are a by-product of amino acid metabolism, they are formed by the spontaneous decarboxylation of intermediate acetohydroxy acids formed during the synthesis of valine and iso-leucine (Krogerus and Gibson, 2013).

1.7 Beer dispense systems

1.7.1 The Cellar

The cellar is a designated space for the storage of beer and other beverages sold by the outlet. As the name suggests they are traditionally located below the bar, however they can be found on the same level or even above the bar in some cases.

Located in the cellar will be beer casks and kegs together with most of the ancillary beer dispense equipment, the keg couplers,

beer lines, fob detectors, remote coolers and in some cases a cleaning ring main. Dispense gas bottles are also stored in the cellar using a stand or chains for attachment to the wall.

Cellar design for many of the older pubs is driven by the architecture of the building and materials of construction. Modern cellars built in completely new buildings can benefit from greater design flexibility. However, little has changed in cellar design and fundamental management. Indeed, Ringwood (1924) notes that all cellar floors and walls should be kept dry with ample drainage built in to the cellar, unnecessary heat should be reduced with the cellar well ventilated and well lit. All of these considerations are still necessary in cellar design today.

Cellars should be kept clean and tidy at all times, spillages should be cleaned up immediately to reduce the chances of harbouring beer spoilage organisms, drains kept clear and beer equipment kept clean. Under the Food Safety Act (1990) accounts can be prosecuted if it is deemed there is a chance for infection of consumers (Nelson *et al.*, 2018).

1.7.2 Cooling

Cellars should be kept at a constant temperature, if cask beer is to be dispensed on the premises, which is highly likely in the U.K., then the cellars are to be kept at 11-13°C. Where kegged beer only is dispensed then the cellar can be chilled to a lower temperature ≈

4°C. Whilst very rare in the UK, cold cellars/rooms are common in the USA and Ireland (Brewers Association, 2019).

As beer travels from the cellar (at 11-13°C) to the tap it needs to be temperature controlled, cask beer should be served at 11-14°C, keg beer at 5-8°C and extra cold products at 1-4°C.

Most of the temperature control is achieved using remote coolers. These coolers have a reservoir of chilled water/ice in which sit stainless steel coils through which beer flows in from the keg and out to the bar. The ice bank provides protection for busy trading periods and provides beer at 5-8°C to the bar. With the trend towards extra cold products, remote coolers using a mix of propylene glycol and water are used. Glycol systems chill below freezing and ensure the delivery of beer at 1-4°C. Where the cellar is > 20 metres from the bar, under-bar cooling systems are required to 'trim' the beer temperature. However, they are not popular with bar staff as they take up space, are noisy and provide heat at the bar (Quain, 2006).

In addition, the remote cooler in the cellar, pumps chilled water/glycol along the length of the beer loom or 'python'. There are two wide bore lines; a forward flow and a return flow. The lines are in contact with the beer lines all the way from the cooler to the bar, with extra cold products located nearest to the them within the loom (see 1.6.5).



Figure 1.6: A remote cooler used to chill beer on route to the bar. A pump is used to send chilled water or glycol along a flow and return line that is in contact with the beer lines housed within the loom or 'python'.

1.7.3 Keg couplers

The keg coupler is the device attached to the cellar end of the beer line, which connects to the keg by clamping to the top of the spear. There is no universal keg coupler with there being as many as six different fittings (Figure 1.7) The mechanism within the keg couplers is however similar to all of the types. It consists of a float control that raises under dispense and then as the tap closes drops back down to prevent back flow of beer from line to keg. There is no current published data about the effectiveness of the float

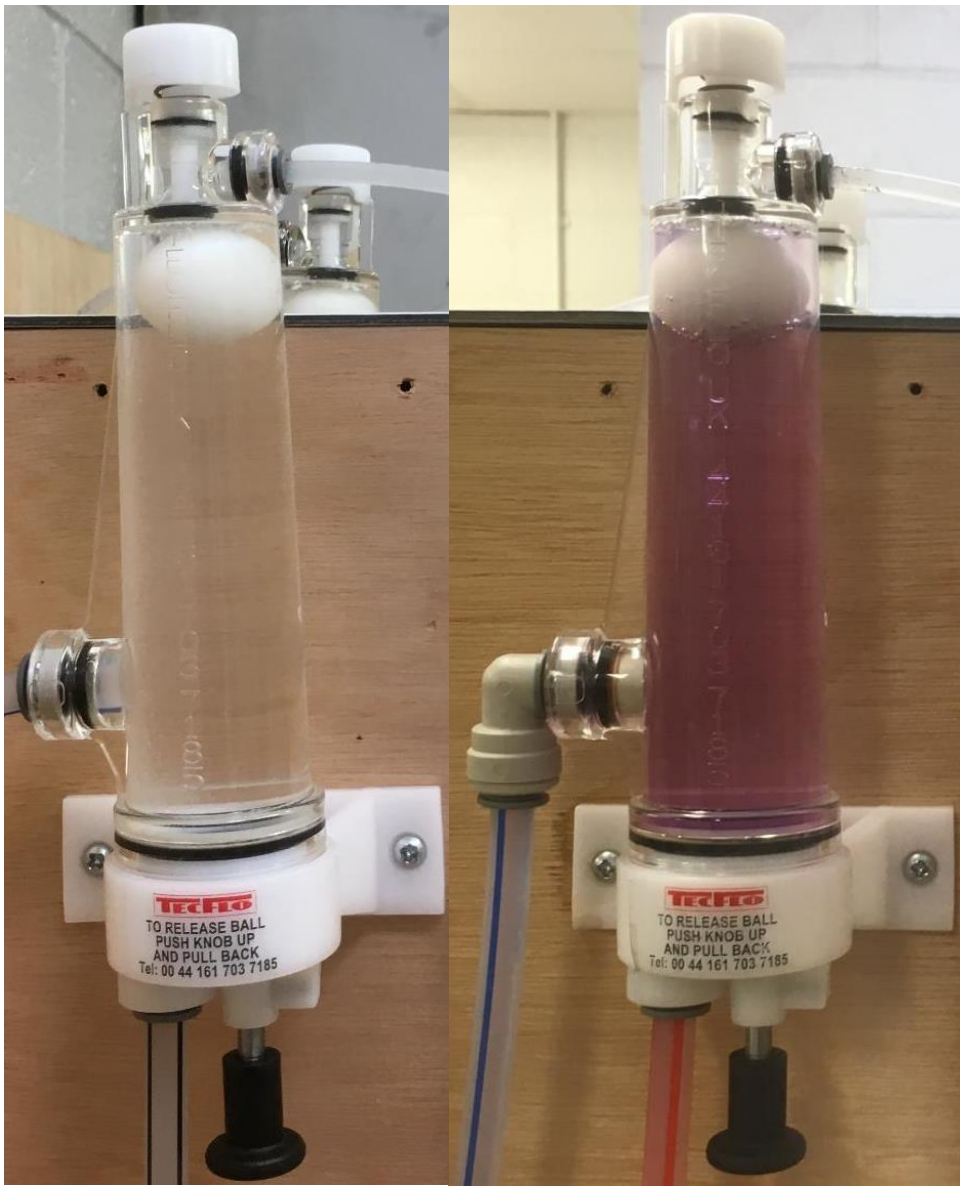
control, and it is considered in this study to be a possible route for keg contamination.



Figure 1.7: Six types of keg spear/coupler combinations. Image sourced from https://www.micromatic.com/keg-couplers/beer-brand-/-keg-taps-couplers-listing?system=u#couplers

1.7.4 Foam on beer (FOB) detectors

Foam on beer (FOB) detectors are located in the cellar between the keg 'drop' line and the remote cooler. The traditional FOB unit (Figure 1.8) contains a float control system whereby upon emptying of a keg during dispense the ball in the chamber drops to block the dispense line. This prevents the dispense line filling with foam, reducing wastage and fobbing.

1340 

1341 **Figure 1.8:** Tec-flo FOB detectors, (L) filled with water, (R) filled with
1342 line cleaner.

1343 Recent innovations have involved moving the FOB to an inline
1344 position, located just above the keg coupler. This design removes
1345 the reservoir of beer and is thought to be more hygienic although
1346 these inline FOBs include internal float controls and again, there is
1347 no available information about the hygienic nature of them.

The Heineken SMART dispense system has gone one step further and removed any internal structure from the fob system and uses sensors to detect beer, air and water (during line cleaning). When the sensor detects air instead of beer it cuts the out the dispense gas so no more liquid/air can be forced out of the keg into the line. The reduction of internal complexity by using sensors instead of float controls within the system, reduces the surface area for microbial attachment and renders the system easier to clean.

1.7.5 Beer lines

Beer lines provide the largest surface area for microbial attachment in beer dispense systems as they can reach lengths of 50 metres (Pawlowsky, 1991). They are often *in situ* for a long period of time (≥ 10 years) (Quain, 2015), due to the complexity of installations, which render replacement practically difficult and financially inviable.

The standard material used for beer lines in the U.K. is medium density polyethylene (MDP). This material took over from PVC in the late 1980s due to its less hazardous nature coupled with improved tensile strength and flexibility (Thomas and Whitham, 1997). Further innovation has focussed on creating a smoother internal surface by the addition of a nylon inner coating. This coating reduces the attachment of microorganisms and subsequent colonisation of the beer line compared to the softer and rougher surface of MDP

(Pawlowsky, 1991). More recent innovations have consisted of reducing gas permeability and thereby maintaining the internal gaseous atmosphere of the beer line. Whilst some investigations into active antimicrobial coatings using silver or molybdenum have been performed on stainless steel for use in the brewery (Priha *et al.*, 2015), the technology if successful may be applicable to beer dispense.

1.7.6 Fonts and Taps

At the ambient end of the dispense system is the font, beer is dispensed from the spout into glassware for consumption by the consumer. Spouts are constructed of stainless steel or plastic (Figure 1.9) and are in most cases removable for cleaning overnight. They are not hygienically designed and can contain many components such as; orifice plates, sparklers and diffusers (Quain, 2015).

They are prone to contamination (Hough *et al.*, 1976; Harper *et al.*, 1980; Storgards and Haikara, 1996) through a variety of routes; human contact, airborne transmission and the environment. Their location, at the warmest site of the dispense system - 'the bar' - renders them particularly vulnerable to contamination by microorganisms.



Figure 1.9: Removable nozzles pictured from two accounts used in this project. Black nozzles are made of plastic and on the right is a stainless-steel version.

Anecdotally, bar staff across the UK (and elsewhere) clean their nozzles by soaking overnight in carbonated 'soda' water in a pint glass/jug. The soda water containing nozzles (including those used to dispense sugar laden soft drinks) spoil overnight with apparent signs of fermentation (turbidity, foam) and the growth of microorganisms on the surfaces of the nozzles. A study by Quain (2016), shows that despite best practice recommending soaking in line cleaner, a better option is to soak tap nozzles in water with a commercial sanitising tablet.

1.7.7 Glassware

The showcase for the product is its appearance in the glass. Glassware is specifically designed for many brands, it can improve appeal and enhance the flavour of the product (Stead *et al.*, 2014; Mirabito *et al.*, 2017). The glass chosen should be clean, undamaged, branded (where appropriate) and with the required nucleation sites at the base to ensure good head retention (Quain, 2006; Nelson *et al.*, 2018).

Glass washers should be used solely for glassware cleaning and not for items such as cigarette trays or coffee cups. This prevents fouling of the glass washer and soiling of glasses. Lipid based substances (milk, cream, lipstick etc) are known to cause foam collapse and soil/rinse aid can create sites of nucleation where bubbles are generated over the internal surface of the glass.

1.8 Line cleaning

The primary defence against poor beer quality is regular and effective line cleaning. This coupled with good hygienic practices around the cellar (keg coupler) and bar (taps and nozzles) would aid in the protection of product quality by reducing the incidence of off flavour, aroma production and clarity issues.

Best practice for line cleaning is recommended to be every seven days in the U.K. whereas this can be extended to 14 -21 days in the U.S.A. and Ireland respectively (Quain, 2015; Nelson *et al.*, 2018;

Brewers Association, 2019). This is likely to be due to their cellars being colder and the prevalence of more effective cleaning by third parties or re-circulating line cleaning machines.

1.8.1 Current line cleaning method

The standard line cleaning process involves emptying the system of beer and flushing all lines with cold water. A solution of a caustic based cleaner diluted in cold water is then pulled into the lines and allowed to sit for anywhere between 15-30 minutes before the dispense of the line volume and re-filling with fresh line cleaner for a further rest. Line cleaning solution is then chased out of the dispense system by copious amounts of water until a neutral pH is achieved. Kegs and casks are then re-connected to the system and beer pulled through.

1.8.2 Line cleaning chemicals

Effective line cleaning compounds should contain an effective detergent for the removal of soils, most commonly line cleaners are found to utilise sodium (or potassium) hydroxide. These caustic based detergents react with proteins and oils changing them into their salt forms making them easier to remove via rinsing.

The cleaning power is improved by the addition of sequestrants which prevent the formation of scale and surfactants which reduce surface tension and allow more effective penetration of the surface by the caustic detergent (Davies *et al.*, 2015).

The sterilant component of most beer line cleaners is sodium hypochlorite, which is a powerful oxidizing agent with broad spectrum anti-microbial activity. Some issues around the use of chlorine based sterilants include the production of chlorophenols and flavour taints due to insufficient rinsing.

Some market leading line cleaners contain an indicator to provide assurance of an effective clean. The use of potassium permanganate, a further oxidising agent - which changes colour upon reaction with biological material - has been used to confirm whether (or not) the clean has been effective. If the line cleaning solution remains purple after a period of rest in the line, then this suggests there is no longer any biological material present in the line and hence cleaning has been successful.

1.8.3 Potential innovations in line cleaning

Effective cleaning requires a combination of time, temperature, chemical action and mechanical action (Boulton and Quain, 2001; Davies *et al.*, 2015). The current line cleaning methodology creates very little mechanical action. Accordingly, the use of re-circulating machines that cause turbulent flow within the dispense system would improve the efficacy of line cleaning (Quain, 2015).

Enzymic pre-treatment of biofilm in beer dispense lines was assessed in a study by Walker *et al.*, (2007). They found that the use of enzymes to degrade the biofilm prior to line cleaning showed

some success. However, variability was found due to the nature of the biofilm present and the specificity of enzymes. They also found that further work needed to be done around the mechanical action of subsequent line cleaning to improve effectiveness.

The Brewers Association in the U.S.A. recommend quarterly acid cleaning with phosphoric or nitric acids to remove the build-up of scale and calcium oxalate as caustic detergents are ineffective at removing inorganic deposits (Davies *et al.*, 2015). They also advocate the use of warm water during line cleaning, this would be expected to improve the effectiveness of caustic based cleaners as their optimal temperature is 40 – 60°C (Singh and Fisher, 2003). The likelihood of this method being adopted in the U.K. however is slim as the availability of hot water in the cellar is rare and anecdotally there are concerns about increasing the temperature within the beer line affecting turnaround time to service.

1.9 Aims and objectives

Beer is an inhospitable environment for many microorganisms. A combination of acidic pH, ethanol, lack of nutrients and hop acids render it a poor medium for microbial growth. However, some yeasts and bacteria can grow in beer causing detrimental changes to flavour, aroma and clarity.

Much work has focussed on beer quality assurance within the brewery setting. Improvements in equipment, effective cleaning in place (CIP) and hygienic design have meant that beer spoilage incidents in the brewhouse and packaging halls have reduced significantly. Control is lost however once the product leaves the brewery. The quality of the final product served to the consumer is in the hands of the accounts serving the consumers.

Little work has been performed on beer dispense and the hygiene of beer dispense systems. The work reported here will generate a greater understanding of the variability of beer quality in the on-trade marketplace.

Improvements can be made by innovations in the construction of beer dispense systems, reducing the level of biofilm formation, microbial loading of beer ex dispense and retention of the aroma and flavour compounds, with the primary goal being to re-define and educate the beer retail industry about beer dispense best practices by quantitatively measuring their beneficial impact on beer quality.

2 MATERIALS AND METHODS

2.1 SAMPLING

2.1.1 'On-trade' accounts

A sample size of 1x half pint measure (284 mL) of draught beer was purchased per brand, per account and samples transferred aseptically into an autoclaved 250 mL Schott Duran bottles.

Samples were transported with cold blocks, in an insulated container to the laboratory. Where they were either stored cold overnight at 4-6 °C, or the forcing test (see method 2.2) was immediately set up.

2.1.2 Dispense rig sampling

In all cases, except where '1st runnings' were required, 120% of the beer line capacity (mL) was pulled through all beer lines prior to sampling (see 2.5.1.1 & 2.5.1.2 for line volumes). This was to replicate as practicably possible the 'real world' scenario and to reduce any bias in terms of dispense line length. 200 mL samples of beer were collected directly into autoclaved Duran bottles and handled as in 2.2.1.

2.1.3 Direct Keg Sampling

To assess any viable microorganisms within the keg, a keg coupler was connected to a stainless-steel pipe and a flow control valve (Figure. 2.1).

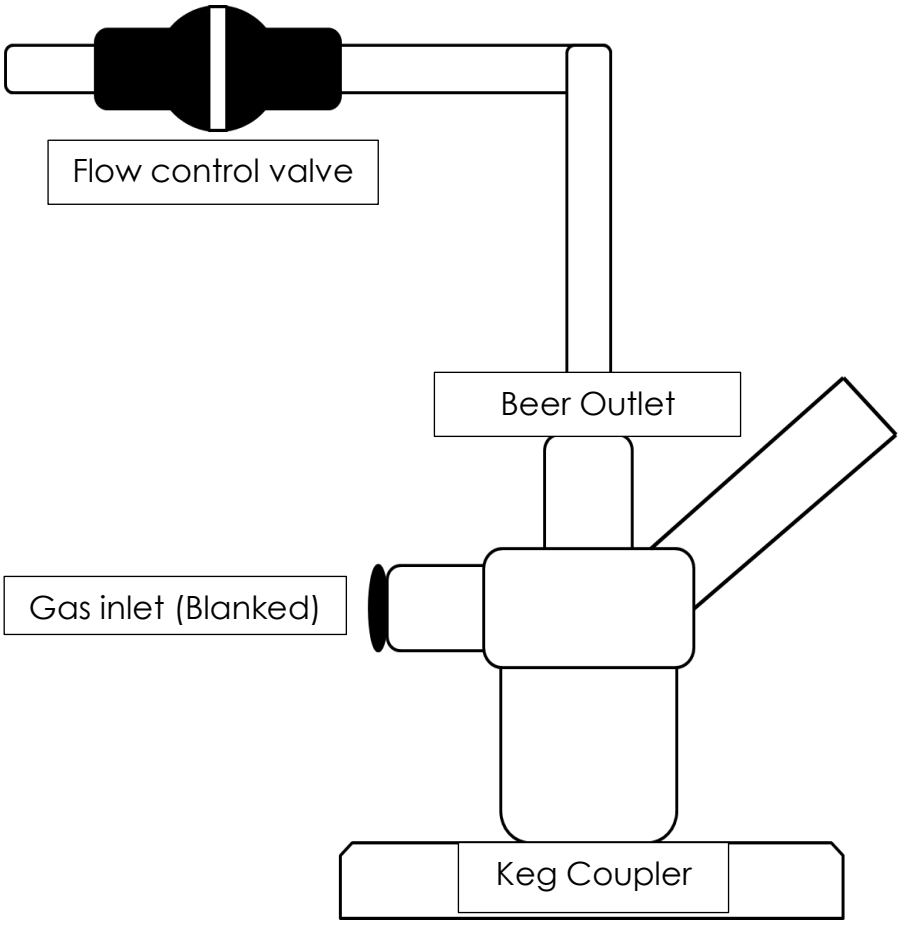


Figure 2.1: Direct keg sampling device comprised of a keg coupler, stainless steel pipe and flow control valve.

Prior to sampling, kegs were shaken to disturb any possible sediment and/or sessile cells to ensure they were resuspended back into solution. The keg coupler and spear head(s) were sanitised with a tap and keg sanitising solution (Chemisphere, UK) prior to connection for sampling.

Once connected the flow control valve was opened and the pressure within the keg enabled a 100 mL sample of beer to be collected directly into autoclaved 250 mL Duran bottles.

Keg beer samples were then assessed using the forcing method outlined in method 2.2.

2.2 Forcing test

A 250 mL sample of beer was split into 3 x 25 mL aliquots by decanting into 30 mL sterile polystyrene universals (STARLAB, UK), the lid was placed on top and left loose to allow for release of pressure and movement of gases during the incubation phase.

Triplicate replicates, 3 x 1 mL aliquots were pipetted into sterile cuvettes (SARSTEDT, catalogue no. 67.742, UK) for absorbance measurement at an optical density of 660 nm (OD_{660nm}) on a Jenway 7315 spectrophotometer. This initial absorbance was recorded for use in calculating the growth of microorganisms.

The 25 mL samples were incubated at 30 °C for 96 hours to enable growth of flora in the beer samples.

Post incubation, samples were 'whirlimixed' and 2 x 1 mL aliquots were pipetted from each universal into sterile cuvettes. Where required, the cuvettes were mixed, and absorbance measured at OD_{660nm} for the 'forced' samples and the readings recorded.

2.2.1 Calculating the Forcing Test Result

To calculate the forcing test result, the initial OD_{660nm} values were subtracted from the final OD_{660nm} values giving the increase in absorbance due to microbial growth, to give ΔOD_{660nm}. The initial

values were subtracted to remove the impact of colour and any initial turbidity of 'hazy' beers e.g. Wheat beer style and un-fined/un-filtered craft beers.

2.2.2 Quality Banding and Quality Index

The scores of the forcing test allowed beers to be placed into a scale of four 'quality bands' based on ΔOD at 660 nm; A – Excellent (ΔOD 0-0.3), B – Acceptable (ΔOD 0.3-0.6), C – Poor (ΔOD 0.06-0.9), D – Unacceptable (ΔOD > 0.9).

The 'quality index' was used to aggregate results for a brand, an account etc. This was calculated by giving each quality band a number; A = 4, B = 3, C = 2 & D = 1. The following formula was then used to provide a % score:

Quality Index (%) = (Total score for all samples/Number of samples) x 100

2.2.3 Differential centrifugation

Post 'forcing', samples were centrifuged to separate the microflora into approximate populations of yeast or bacteria (Catsimpoolas, 2012).

To pelletize the yeast, an Initial centrifugation at 500 x g (Rech *et al.*, 1990) for 5 mins was performed and the supernatant decanted into a second sterile Universal which was then centrifuged at 3000 x g (Niederhauser *et al.*, 1992) for 10 mins to pelletize bacterial cells.

Measurement of the OD_{660nm} of the supernatant was recorded after each spin to calculate the relative population contribution to total OD increase within a sample. I.e. % Yeast and % Bacteria.

2.3 MICROBIOLOGICAL MEDIA AND CULTURE

Plates were prepared for the study of microflora and growth of colonies for subsequent use in genomic evaluation. All media was supplied by (Oxoid UK, Thermofisher Scientific unless stated) and autoclaved at 121 °C for 15 mins at a pressure of 15 psi prior to plates being poured.

2.3.1 Wallerstein Laboratories Nutrient Agar Media (WLN)

WLN media has been commonly used in the brewing industry for the detection of wild yeasts, both *Saccharomyces* and non-*Saccharomyces*. The inclusion of the pH indicator bromocresol green at 22 mg/L aids in the identification of yeasts due to the different pH of the microbial colonies.

WLN media powder (for composition see Table 2.1) was added to RO (reverse osmosis treated) water at a rate of 75 g/L and thoroughly mixed prior to autoclaving (Jespersen and Jakobsen, 1996).

2.3.2 Wallerstein Laboratories Differential Agar Media (WLD)

WLD media was prepared by following the procedure for WLN (2.3.1) with the addition (post autoclaving) of 0.1% (w/v)

cycloheximide solution (Sigma Aldrich, UK) at a rate of 4 mg/L to suppress yeast growth.

Table 2.1 Composition of WLN/WLD media.

Component	g/L
Yeast extract	4.0
Tryptone	5.0
Glucose	50.0
Potassium dihydrogen phosphate	0.55
Potassium chloride	0.425
Calcium chloride	0.125
Magnesium sulphate	0.125
Ferric chloride	0.0025
Manganese sulphate	0.0025
Bromocresol Green	0.022
Agar	15.0

2.3.3 Raka Ray Media

Raka Ray media made to the formulation developed by (Saha, Sondag and Middlekauff, 1974) is recommended by both the American Society of Brewing Chemists (ASBC) and European Brewing Convention (EBC) for the detection and culture of lactic acid bacteria.

Plates were prepared by addition of Raka Ray powder (Table 2.2) to RO water at a rate of 77.1 g/L. Additions post autoclaving of Tween 80 (sorbitan mono-oleate) (Sigma Aldrich, UK) at 10 mL/L

and cycloheximide solution (0.1%, w/v) (Sigma Aldrich, UK) at 7 mL/L, render the media selective for lactic acid bacteria.

Table 2.2 Composition of Raka Ray media.

Component	g/L
Yeast extract	5.0
Tryptone	20.0
Liver concentrate	1.0
Maltose	10.0
Fructose	5.0
Glucose	5.0
Betaine HCL	2.0
Diammonium hydrogen-citrate	2.0
L-aspartic acid	2.5
L-glutamic acid	2.5
Magnesium sulphate	2.0
Manganese (II) sulphate	0.66
Potassium phosphate	2.0
N-acetyl glucosamine	0.5
Agar	17.0

2.3.4 Plate Count Media

Plate count agar is a commonly used media for the plate counting of food stuffs (Pattison *et al.,* 1998; Valadez-Blanco *et al.*, 2012) plates were produced by the addition of 17.5 g of media powder (Table 2.3) to 1 L of RO water, followed by thorough mixing and autoclaving as above.

Table 2.3 Composition of PCA media.

Component	g/L
Yeast extract	2.5
Tryptone	5.0
Glucose	1.0
Agar	9.0

2.3.5 YPD Media

YPD media consisting of 1% (w/v) yeast extract, 2% (w/v) D-glucose (Sigma Aldrich, UK) and 2% (w/v) bacteriological peptone (Sigma Aldrich, UK) was used for the growth of selected laboratory strain organisms.

2.3.6 Incubation

All media except Raka-Ray, were incubated aerobically at 30 °C for 2-5 days. Raka-Ray plates were incubated in 2 L anaerobic jars using Anaerogen packs (Oxoid, UK) to create an anaerobic atmosphere suitable for the growth of anaerobes, predominantly the lactic acid bacteria species. Again Raka-Ray plates were incubated at 30 °C for between 5-7 days to allow for slower growing organisms.

2.3.7 Plating

In all cases, a 100 µL sample of the required dilution was pipetted on to media plates and spread using sterile individually wrapped L-shaped spreaders. Where the colony count was to be recorded,

triplicate plating and serial dilution to a countable range of 30-300 colonies was performed.

2.3.8 TECAN

For further validation of the forcing test method developed in Chapter 3, validated laboratory microorganisms (Table 2.4) were grown and their growth measured in three brands of beer, each of a different beer style; Carling (Lager), Guinness (Stout) and Worthington (Ale) and two ciders; Strongbow and Strongbow Dark Fruit.

Table 2.4 Laboratory organisms used during TECAN experiment.

Organism (Strain)	**Information**
Saccharomyces cerevisiae (1332)	Ale yeast
Saccharomyces pastorianus (W34)	Lager yeast
Brettanomyces anomalus	Wild yeast
Lactobacillus brevis	Lactic acid bacteria
Pediococcus damnosus	Lactic acid bacteria

Growth was tracked by the addition of each organism individually, to each brand in triplicate on a 96-well microtiter plate.

Initially, each organism was grown in YPD media (section 2.3.5) to an OD_{660nm} of 1.000, 100 µL of this growth stock was then pipetted into a sterile 30 mL universal container which contained, 25 mL of

each of the brands included in the experiment. From this universal 200 µL was then added to a well on the microtiter plate.

Cell concentration was tracked optically at OD_{660nm} (in accordance with method 2.2) by an automated plate reader, TECAN (Infinite ® PRO series, TECAN, UK). Readings were taken every 30 minutes for a total of 120 hours. The data was collected and analysed by Magellan™ Data analysis software (TECAN, UK).

2.4 GENOMIC ANALYSIS OF MICROORGANISMS IN DRAUGHT BEER

To better understand the microflora of draught beer, molecular methods were employed to identify the organisms present.

2.4.1 DNA Extraction

2.4.1.1 Lithium Acetate Extraction

For extraction of yeast DNA, the method described in (Lõoke, Kristjuhan and Kristjuhan, 2017) was used. A 200 mM lithium acetate (LiOAc), 1% (w/v) Sodium dodecyl sulphate (SDS) lysis solution was prepared and filter sterilised for use in the extraction.

100 µL of lysis solution was pipetted into sterile Eppendorf tubes (1.6 mL) added to which was either; a colony of yeast from a media plate or, 200 µL of a liquid culture. Tubes were then heated to 70 °C for 5 mins using a heat block. 300 µL of absolute ethanol (96-100%) (Sigma Aldrich, UK) was added and the mixture vortexed. Samples were centrifuged at 15000 x g for 3 mins to pelletise the

DNA and cell debris, the supernatant was poured off and the pellet re-suspended with 300 µL of 70% (v/v) ethanol and the centrifugation step repeated. Again, the supernatant was poured off and the pellet washed with 70% (v/v) ethanol, following this the pellet was dissolved in 100 µL of Tris-EDTA (10 mM Tris, 1 mM EDTA) pH 7.6 buffer (TE buffer) (Sigma Aldrich, UK) and any remaining cell debris pelletised by a final centrifugation at 15000 x g for 15 seconds.

2.4.1.2 Phenol-Chloroform Extraction

For extraction of bacterial DNA, the method of (Legras and Karst, 2003) was used. Single bacterial colonies, grown on plates (Section 2.3) were picked using sterile individually wrapped loops and placed into 2 mL cryovials (Sarstedt, UK) and suspended in 400 µL lysis buffer (10 mM Tris pH 7.6, 1 mM EDTA, 100 mM NaCl, 2% (w/v) Tritox X (v/v)(Sigma Aldrich, UK) and 0.5 g glass beads (0.45 – 0.55 mm diameter, acid washed) (Sigma Aldrich, UK) were added to the cell suspension which was vortexed for 4 minutes. A further addition post-vortex of 200 µL of TE buffer was added before centrifuging for 5 minutes at 4,000 x g. The upper phase was then pipetted into a sterile 1.6 mL Eppendorf tube and combined with 500 µL of chloroform/isoamyl alcohol 98/2 (v/v). Samples were then mixed by inversion and then centrifuged at 22,000 x g for 2 minutes. The aqueous phase was pipetted into a sterile 1.6 mL Eppendorf

tube on ice and two volumes of absolute ethanol (96-100%, v/v) (Sigma Aldrich, UK) added to precipitate DNA. A final centrifugation step of 22,000 x g for 5 minutes pelletised the nuclear DNA and post-centrifuge the supernatant was discarded, and the pellet allowed to air dry on the bench-top for 15 minutes. The resultant pellet was then suspended in 50 µL of 10mM TE buffer at pH 8.0 (Thermofisher Scientific, UK) and either used immediately or stored at -20 °C for future use.

2.4.2 DNA Amplification

Extracted DNA (see 1.4.1) was amplified in preparation for purification and sequencing, in order to identify the microorganisms growing within draught beer.

PCR reactions were carried out in 0.5 mL microcentrifuge tubes and the total reaction volume was 50 µL. The reaction mixture for each sample is detailed in Table 2.5.

All DNA samples were amplified using a Techne TC-512 (Cole Palmer, UK) thermocycler using the following conditions; Initial denaturation at 94 °C for 5 min, 34 cycles of denaturing for 30 s at 92 °C, annealing for 45 s at 54 °C with an extension for 75 s at 72 °C followed by a final extension phase of 5 min at 72 °C. Upon completion of the cycle the thermocycler held samples at 4 °C.

Table 2.5 PCR Reaction tube mixture

Component (Stock Concentration)	Volume (µL) per 50 µL reaction	Final Concentration
ddH_2O	30.4 µL	N/A
Buffer (5X) (NEB, UK)	10 µL	1X
MgCl2 (50 mM)	1.5 µL	1.5 mM
dNTP (10 mM)	1.0 µL	200 µM
Forward primer (10 µM)	2.5 µL	500 nM
Reverse primer (10 µM)	2.5 µL	500 nM
Taq Polymerase (NEB, UK)	0.1 µL	
Sample DNA	2 µL	

All PCR products were visualised on 1.0% (w/v) agarose gels (Sigma Aldrich, UK) containing 3 µL ethidium bromide (Invitrogen, UK) at a concentration of 0.2 µg/mL using gel electrophoresis at 90 mV for 60 minutes.

DNA bands were viewed under UV light using a GelDoc-IT ® 2 Imaging system (UVP LLC, USA), bands were run alongside a 100 bp ladder (Promega, UK).

2.4.2.1 Internal Transcribed Spacer Region (ITS) PCR

The internal transcribed spacer region (ITS) is common to fungi and has been used to identify yeasts to the species level. This region

spans two spacers known as ITS 1 and ITS 2 and a 5.8S rRNA gene (Valente *et al.*, 1996; Guillamön *et al.*, 1998; Montrocher *et al.*, 1998; Esteve-Zarzoso *et al.*, 2009; Pham *et al.*, 2011). The primers (Higuchi, 1990) for ITS PCR are shown in Table 2.6. once visualised on agarose the band sizes provided an early estimation of species diversity by comparison with the tables reported by Pham *et al.*, 2011.

Table 2.6 ITS PCR primer sequences.

Name	**Sequence**
ITS1	5'-TCCGTAGGTGAACCTGCGG-3'
ITS4	5'TCCTCCGCTTATTTGATATGC-3'

2.4.2.2 16S PCR

For bacteria, the 16S ribosomal DNA (rDNA) was amplified, as with the ITS region in yeasts, the 16S rDNA is conserved amongst bacterial species and has been used to identify bacteria in mixed populations (Marchesi *et al.*, 1998; Zhu *et al.*, 2002). Table 2.7 describes the primers used for 16S PCR (Marchesi *et al.*, 1998)

Table 2.7 16S PCR primer sequences.

Name	**Sequence**
63F	5'-CAGGCCTAACACATGCAAGTC-3'
1387R	5'-GGGCGGWGTGTACAAGGC-3'

2.4.3 DNA Purification

Amplified DNA was purified for sequencing and subsequent species identification, using a PCR purification kit (PP-201L, Jena Bioscience, Germany).

The purified product was acquired after a binding phase, multiple washes and then a final elution step. Prior to starting, the washing buffer is diluted to a final volume of 200 mL with absolute ethanol (96-99%) (Sigma Aldrich, UK).

Initially 250 µL of binding buffer was added to 50 µL of DNA and mixed well. Following this, the spin columns were prepared for sample addition. A spin column was inserted inside a 2 mL collection tube and 100 µL of activation buffer pipetted onto the spin column membrane. The tubes were then centrifuged at 10,000 x g for 30 s.

The DNA samples were then added to the activated spin columns and a further centrifugation at 10,000 x g for 30 sec performed. Liquid in the base of the collection tube was discarded post centrifugation.

The spin columns were loaded with sample DNA and the following wash step was performed twice. A wash involved adding 700 µL of washing buffer to the spin columns and centrifuging again at 10,000 x g for 30 s. Again, the liquid that had flowed through the spin column was discarded. To complete the wash a final

centrifugation at 10,000 x g for 2 minutes was performed with any flow-through discarded.

Finally, the elution of the purified DNA product involved placing the washed spin columns into fresh sterile 1.6 mL Eppendorf tubes and an addition of 50 µL of elution buffer directly onto the spin column membrane. The tubes were then incubated for 1 minute at room temperature (20-24 °C) before centrifugation at 10,000 x g for 1 minute to elute the DNA.

Purified DNA was stored at -20 °C prior to sequencing.

2.4.4 DNA Sequencing

Purified DNA amplicons were prepared and sent for sequencing by Source Bioscience, UK, who performed all sequencing activities. Upon reception of the DNA sequences, these were entered in the NCBI BLAST database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for genus and species identification.

2.5 Hygienic assessment of dispense systems

2.5.1 Dispense system(s)

Bespoke dispense systems were designed and installed alongside Diageo GB and Molson Coors UK.

All dispense lines were supplied by Valpar, Micro Matic U.K. and the following dispense line types were used:

MDP – Medium density polyethylene

Brewmaster 2 – Is similar to an MDP line but with a smoother inner surface caused by an internal nylon coating.

Barriermaster Flavourlock – Constructed in a novel way to reduce gas permeability across the beer line coupled with the smooth surface nylon coating as seen in Brewmaster 2 line.

2.5.1.1 Diageo Dispense Rig

Table 2.8 Diageo dispense rig setup.

Line Code	Line Material	Line Volume (mL)	Cooler	FOB Type
G1/G2 H1/H2	MDP	1800	Remote	Y – Tec-flo
G3/G4 H3/H4	Barriermaster	1800	Remote	Y – Tec-flo
G5/G6 H5/H6	Brewmaster 2	1800	Remote	Y – Tec-flo
G7/H7	Barriermaster	600	2-line cooler	Y – Inline
G8/H8	Barriermaster	600	2-line cooler	None
G9/H9	MDP	600	4-line cooler	G - inline H - None
G10/H10	Barriermaster	600	4-line cooler	G – inline H – None

G = Guinness lines, H = Hop House 13 lines

2.5.1.2 Molson Coors Dispense Rig v1

Note: All beer lines on Molson Coors dispense configurations had a line volume of 1800 mL

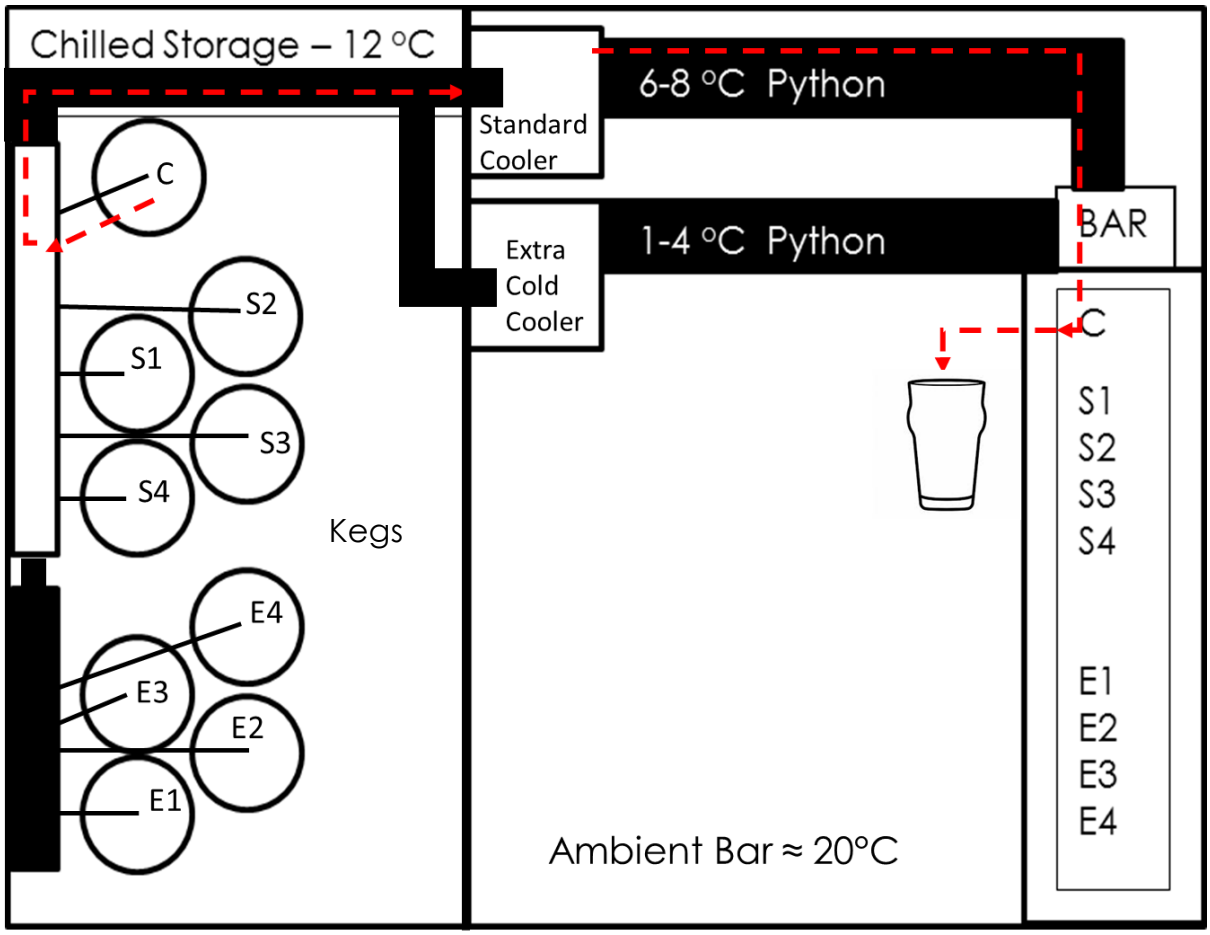


Figure 2.2: Schematic of the dispense system designed and installed by Molson Coors UK at the University of Nottingham. - - - - indicates flow of beer from keg through to glass.

Table 2.9 Molson Coors dispense system setup.

Line Code	Line Material	Tap Cooling	Temperature
Control	MDP	Trace	Standard 6-8°C
S1	Barriermaster	Re-Circulation[1]	Standard 6-8°C
S2	Brewmaster 2	Re-Circulation	Standard 6-8°C
S3	Barriermaster	Trace[2]	Standard 6-8°C
S4	Brewmaster 2	Trace	Standard 6-8°C
E1	Barriermaster	Re-Circulation	Extra Cold 1-4°C
E2	Brewmaster 2	Re-Circulation	Extra Cold 1-4°C
E3	Barriermaster	Trace	Extra Cold 1-4°C
E4	Brewmaster 2	Trace	Extra Cold 1-4°C

1. Re-circulation cooling is defined as, circulation of chilled water along the entirety of the system including inside the font body and to the back of the dispensing tap.
2. Trace cooling defined as chilled water circulation from remote cooler to base of the font only.

2.5.1.3 Molson Coors Dispense Rig v2

Once all experiments required were completed on version 1 of the dispense system, the tap cooling method was changed so that all extra cold lines had re-circulating cooling to the taps. The line cleaning trial was performed on lines S1-S4 and line cleaning frequency extension trials continued on extra cold lines only.

Table 2.10 Molson Coors dispense system after changes made.

Line Code	Line Material	Tap Cooling	Temperature
Control	MDP	Trace	Standard 6-8°C
S1	Barriermaster	Trace	Standard 6-8°C
S2	Brewmaster 2	Trace	Standard 6-8°C
S3	Barriermaster	Trace	Standard 6-8°C
S4	Brewmaster 2	Trace	Standard 6-8°C
E1	Barriermaster	Re-Circulation	Extra Cold 1-4°C
E2	Brewmaster 2	Re-Circulation	Extra Cold 1-4°C
E3	Barriermaster	Re-Circulation	Extra Cold 1-4°C
E4	Brewmaster 2	Re-Circulation	Extra Cold 1-4°C

2.5.2 Contamination of dispense systems

2.5.2.1 Production of contaminated beer

Initial contamination of the bespoke dispense system(s) was undertaken to improve the reliability of the results when compared with future trials in the 'on-trade'. The dispense rigs being evaluated had new unspoiled and un-damaged beer lines and hence would not have been representative of dispense systems in use which can have seen 10+ years of use.

Beer samples of the brand to be tested were taken from the 'on-trade' as in 2.1.1, a combination of samples from a total of six accounts (6 x 250 mL) were then combined into a 2 L Duran bottle

for incubation at 30 °C for 96 hours. After this initial incubation the beer was thoroughly mixed by inversion to resuspend sedimented microorganisms. Fresh media, a 440 mL can of the same brand was added to the Duran bottle to give a total volume of 2 L. Further incubation for 72 hours at 30 °C, ensured ample microbial growth

2.5.2.2 Dilution and inoculation of contaminated beer

The contaminated beer sample produced in 2.5.2.1 were mixed by shaking and inversion prior to dilution with fresh beer (of the same brand) from a previously unopened keg at a rate of 1 L contaminated beer to 9 L of fresh beer.

This process was performed in a 10 L capacity, pressurised cleaning bottle (Micromatic, USA) and was coupled to each line and used to transfer the contaminated beer throughout the dispense system. The addition to each line of line volume + 20% provided an equivalent amount of contaminated beer to each line.

2.5.2.3 Incubation of contaminated beer in dispense system

The dispense system was left at room temperature with no cooling applied for a period of 14 days to encourage microbial growth in the system.

Fresh kegs were connected to each line post contamination and beer pulled through once every three days, this reduced the turbulence in the system allowing for the settlement of microorganisms and initiation of biofilm formation. Again, the

sample volume of line volume + 20% was removed each time to provide fresh media to the microorganisms within the system.

At the end of the 14-day period every line was assessed by the forcing method with results in the 'C' to 'D' quality bands (see 2.2.2) confirming that the system was heavily contaminated.

2.5.3 Trade throughput matching

Beer throughputs were matched to a typical weekly scenario in the on-trade. 1 Keg (50 L) of beer was dispensed through each line each week as per Table 2.11

Table 2.11 Beer throughputs per week.

Day	Trade Day	Morning Volume (L)	Afternoon Volume (L)	Total Volume (L)
Monday	Wednesday	2.5	2.5	5
Tuesday	Thursday	3.5	3.5	7
Wednesday	Friday	5	5	10
Thursday	Saturday	4.5	4.5	9
Friday	Sunday	4	4	8
Saturday	Monday	2	2	4
Sunday	Tuesday	3	3	6

2.5.4 ATP bioluminescence swabbing of components

ATP surface swabs (3M™ Clean-Trace™, UK) were used to measure the hygienic status of dispense tap nozzles and keg couplers.

Swabbing was performed by moving a fresh swab in two directions across the surface of the component and placing it into the reaction tube. Here, it combines with luciferase causing the oxidation of the substrate luciferin and a subsequent light emission that was measured using the 3M NG Luminometer giving a result in RLU (Relative light units).

2.5.4.1 Biofilm measurement

Segments of beer line were periodically removed for the measurement of biofilm.

Beer lines were cut at the point of exit from the remote cooler, this site was selected for its accessibility and because it was located outside of the cellar, therefore unaffected by any further external temperature management.

For 'pre-cleaning' samples, flow valves were closed, and the lines drained to remove any residual beer prior to taking samples. 'Post cleaning' samples were taken after the full cleaning cycle (see method 2.5.5) had been performed and prior to re-connection of beer kegs and subsequent pull through of beer. The segments of

beer line were collected in 30 mL sterile polystyrene universal containers (STARLAB, UK).

The segments were washed, pipetting 20 mL of sterile RO-water into each universal and vortexed for 30 seconds to remove loosely associated cells from the beer line. The absorbance of the rinse water was measured at OD_{660nm}.

Beer line segments were swabbed using transport swabs (Sterilin eSwabs, Appleton Woods, UK) that were moistened by dipping into sterile RO-water. Swabbing was performed in two directions to maximise the pickup of cells and the swabs placed into a tube containing 1 mL of Amies solution (Table 2.12) (Percival *et al.*, 1998; Hallam *et al.*, 2001; Fielding *et al*., 2007).

The tubes were vortexed for 30 seconds to transfer cells from the swabs into solution. The OD_{660nm} of the Amies solution was then read to determine loading from the swabs.

Table 2.12 Composition of Amies transport medium.

Component	g/L
Charcoal (pharmaceutical)	10.0
Sodium chloride	3.0
Sodium hydrogen phosphate	1.15
Potassium dihydrogen phosphate	0.2
Potassium chloride	0.2
Sodium thioglycolate	1.0
Calcium chloride	0.1
Magnesium chloride	0.1
Agar	4.0

2.5.5 Line cleaning

To clean the dispense systems the following standard trade protocol by the BII (British Institute of Innkeeping) was used:

- All beer lines connected to a water source either through cleaning main or wash bottle
- All taps emptied of beer until water observed from spout
- Correct volume of beer line cleaner diluted in cold water (according to manufacturer's guidelines)
- Line cleaner pulled through each tap until cleaner observed coming from spout. If the line cleaner contains no indicator, litmus papers are used to check pH

- After a 15-minute rest, a fresh volume of line cleaner is pulled through each tap
- After a further 15-minute rest all line are emptied of line cleaner and flushed through with copious amounts of water until either; no colour is detected in liquid coming from spout or, a neutral pH is achieved
- All lines reconnected to kegs and beer pulled through until it is observed from spout

Keg couplers were disconnected from the kegs and attached to cleaning bottles (Micromatic, USA) containing cold water. All taps were then opened and drained of beer until water was coming from the tap.

Cleaning bottles were then filled with beer line cleaner (diluted in cold tap water). See Table 2.13 for dilution rates and soak times of individual cleaners.

Dispense lines were filled with line cleaning detergent by dispense of liquid until a solid stream of line cleaner from the tap. FOB (Foam on beer) detectors were bled to ensure they were full of line cleaner (Figure 2.3)

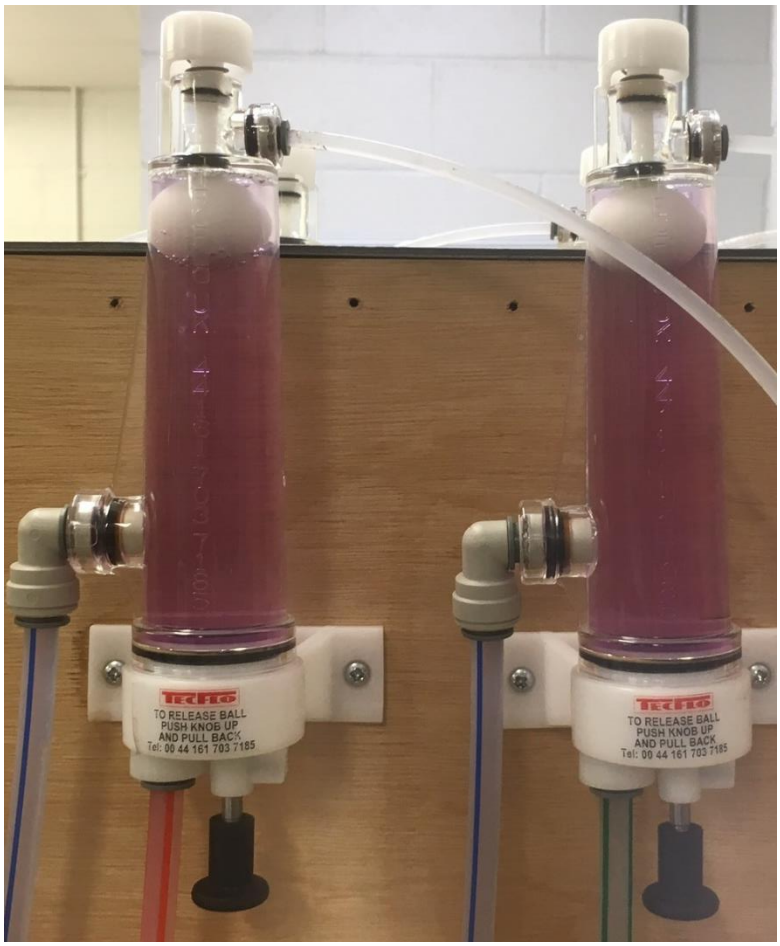


Figure 2.3: Tec-Flo foam on beer (FOB) detectors, fully filled with line cleaner during cleaning cycle.

Unless otherwise stated in Table 2.13, a standard soak time of 15 minutes was used for line cleaning. After the initial soak the full line volume + 20% was 'pulled through', with fresh beer line cleaner for the second and, where required, third soak periods.

Once all soak periods were completed, the lines were rinsed with fresh cold tap water. Where the line cleaning solutions included a colour indicator, water was rinsed through until colourless. With colourless line cleaners, litmus paper was used to measure pH with rinsing complete when pH 6.0-8.0 was attained.

Measurement of the volume of liquid rinsed through was recorded to ensure no line received additional cleaning or turbulent flow.

Once cleaning had been completed, keg couplers and keg spears were sprayed with a sanitising spray (Chemisphere, UK) prior to reconnection. All lines were then opened and line volume + 20% drained until a steady stream of beer was observed from the tap.

2000 **Table 2.13** Line cleaners, respective dilutions and soak times.

Line cleaner	Active ingredient	Manufacturer	Dilution rate (mL/L)	Soak time (minutes)	Number of Soaks
Zenith	Sodium hydroxide (5-15% w/w) Sodium hypochlorite (<5% w/w)	Zenith hygiene group	25	15	3
Pipeline Original	Potassium hydroxide (<5% w/w) Sodium hypochlorite (<3% w/w) Sodium carbonate (<20% w/w)	Chemisphere	25	5	Until purple colour seen
Prosan Plus	Sodium hydroxide (1-10%) Sodium hypochlorite (1-10%) Potassium permanganate (<1%)	Proton	10	10	Max 3
Desana Verify	Potassium hydroxide (10-25% w/w) Sodium hypochlorite (1-5% w/w) Sodium permanganate (% w/w)	Thonhauser (Avani Solutions)	33	15	5-minute soaks until purple colour seen
Full Clear	Enzyme mix	Full Clear UK	20	120-240	2

2001

Manufacturer's details:

- Zenith Hygiene Group PLC, Hatfield, Hertfordshire, U.K.
- Chemisphere UK Ltd, Kettering, Northamptonshire, U.K.
- The Proton Group, Normanton, West Yorkshire, U.K.
- Thonhauser GmBH, Giesshubl, Austria
- Full Clear UK, Leeds, West Yorkshire, U.K.

2.6 ANALYSIS OF AROMA AND FLAVOUR COMPOUNDS IN DRAUGHT BEER

To assess the impact of hygiene on beer quality, beer volatiles and vicinal diketone (VDK) levels were measured at various points between cleaning and over different dispense times. Microorganisms are known to metabolise and produce various flavour and aroma compounds which affect the quality of beer (Wainwright, 1973; Peddie, 1990; Suzuki, 2011; Ridgway, 2015).

All samples were run in triplicate and in a random sequence to reduce impact of machine drift or error.

2.6.1 Beer volatiles

Headspace gas chromatography (HS-GC) was performed using the method recommended in the Analytica of the European brewing convention (Analytica EBC) method number 9.39 (2000).

2.6.1.1 Standard Curve Production

A standard curve was generated using known standards prior to each run of analysis. See Table 2.14 for each compound and the concentrations prepared.

Table 2.14 Beer volatile compounds and their standard concentrations.

Compound	Concentration (mg/L)
3-methyl-1-butanol	10-100
Isobutanol	5-50
Propanol	5-50
Acetaldehyde	10-100
Isoamyl acetate	0.5-5
Ethyl acetate	10-100
Ethyl hexanoate	0.1-1
Ethyl octanoate	0.1-1

2.6.1.2 Quantification of beer volatile compounds

Beer samples were stored at -20 °C post collection and thawed at 4 °C prior to degassing for measurement on the GC. Samples were degassed by sonication in an ultrasonic water bath for 5 minutes.

10 mL of degassed beer was pipetted into a HS-GC vial (Thermofisher Scientific, UK) and 50 µL of an internal standard added. The internal standard was prepared from 0.62 mL of 1-

butanol diluted in absolute ethanol to 50 mL giving a final concentration of 1.6 mg/L. Sodium chloride (3.5 g) was added and vials crimp sealed with a GC vial cap (Sigma Aldrich, UK).

Volatiles were measured using a Scion 456-Gas Chromatograph (Scion instruments, West Lothian, UK), 500 µL of sample was injected using a PAL Combi-XT autosampler (PAL System, Zwingen, Switzerland) onto a Zebron ZB Wax column (60 m x 0.25 ID, Phenomenex Inc, Cheshire, UK). The carrier gas was helium delivered at a constant pressure of 15 psi. The chromatographic run started with an initial oven temperature of 85 ˚C for 10 minutes, followed by ramping up of temperature at a rate of 25 ˚C /minute to 110 ˚C for a further 13 minutes, before increasing up to 200 ˚C at a rate of 8 ˚C/minute for 13.25 minutes.

Results were recorded and quantified in mg/L by a software package, Chromeleon ™ Chromatography Data System (CDS) (Thermofisher Scientific, UK)

2.6.2 Vicinal Diketone

Another set of compounds produced by beer spoilage microorganisms are the vicinal diketones, most commonly 2,3-butanedione known as diacetyl (Krogerus and Gibson, 2013). Quantification of VDK was performed using headspace gas chromatography (HS-GC) using method 9.24.2 of the Analytica-

EBC (1999). A standard curve was prepared prior to each run, see Table 2.15 for the compounds and concentrations used.

Table 2.15 Compounds measured.

Compound	Concentration (mg/L)
2,3-butanedione (diacetyl)	0-25
2,3-pentanedione	0-25

Beer samples were stored and de-gassed as for beer volatile analysis. Sample (5 mL) was pipetted into a HS-GC vial, with the addition of 50 µL of internal standard. The internal standard was prepared from a 5 mg/L solution of 2,3-hexanedione prepared by diluting 268 µL of 2,3-hexanedione to 100 mL with absolute ethanol. 0.5 mL of this solution was diluted to 250 mL with RO-water. Ammonium sulphate (3.5 g) was added and each vial crimp sealed immediately.

Sample (500 µL) was injected as in 2.6.1.2 onto a Restek Rtx-5MS (Crossbound 5% diphenyl/ 95% dimethyl polysiloxane) 30 m, 0.25 mm ID, 0.8 µm df column. (Restek, USA). The carrier gas was helium delivered at a constant pressure of 50 psi.

Then initial oven temperature was 30 °C for 2 minutes which was increased at a rate of 70 °C /minute to 120 °C for 2 minutes and then increased at a rate of 8 °C /minute to 200 °C for 13.25 minutes.

Results were recorded and quantified using Chromeleon CDS software.

3 DEVELOPMENT OF A BEER QUALITY FORCING TEST

Foreword

The initial phase of the project was focussed on the development of a simple, effective and indicative test for on-trade beer quality. Allowing for the classification of beer quality into four quality bands at the point of dispense.

The method and subsequent validation work presented in this chapter were first published in the Journal of the Institute of Brewing on the 29th of November 2017.

My contribution to this paper consisted of being lead author and producer of data presented forthwith under the supervision of Dr David Quain and aided with sample microscopy by Melanie Stuart.

Supplementary information for the paper is included in Appendix I whilst, further data in support of section 3.3.2 can be seen in Appendix II.

Draught beer hygiene: a forcing test to assess quality

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Abstract

The quality of draught beer is important to consumers but can be inconsistent, ranging from excellent through to unacceptable. The few but dated studies of draught beer quality have focused on the number of microorganisms that are present in the product. Work reported here, suggests that this approach has its limitations and fails to relate to beer quality post-dispense. An alternative approach using the long-established 'forcing' method provides a better but still retrospective assessment of draught beer quality. Samples post dispense are 'forced' by static incubation at 30°C for four days and beer quality is ranked by the measurement of absorbance at 660nm. The increase in absorbance reflects the

growth of beer spoilage microorganisms present in the beer at dispense. Four quality bands are proposed, where quality is described as excellent (absorbance increase of < 0.3), acceptable (0.3-0.6), poor (0.6-0.9) and unacceptable (> 0.9). The method is straightforward, requires no special skills and enables, for the first time, the robust quantification of draught beer quality. It is anticipated that the method will have widespread application in the measurement and improvement of the quality of draught beer.

Additional supporting information can be found in the online version of this article at the publisher's website.

Keywords: dispense; beer spoilage; quality; method

Short title: A simple method to assess draught beer quality

3.1 Introduction

The mix between the on-trade/on-premise (pubs, bars and restaurants) and the off-trade/off-premise (supermarkets, shops) varies widely across the world. In 2014 *(1)*, the major on-trade markets include Ireland and Spain (64%), the UK (50%), Japan and South Korea (48%) with the Czech Republic, Italy, Belgium and Australia accounting for 40-45%. Globally, draught beer in on-trade licensed premises accounts for 7% or more of the worldwide market although, in most countries, volumes are either static or in decline *(2)*. This reflects a host of factors – political, economic,

social and technological - that impact the on-trade *(3)*. Of these, poor or compromised draught beer quality is an important consideration, which in turn, is exacerbated by comparatively high pricing.

For beer, quality has been defined as 'meeting the customer requirements' *(4)* and 'the achievement of consistency and elimination of unwanted surprises' *(5)*. More specifically, from the perspective of consumers *(6)*, beer quality reflects parameters such as colour, aroma, alcohol content, haze, foam, flavour and gas content. For beers packaged into bottle and especially can, such criteria are broadly stable. However, draught beer in kegs and cask, which - on packaging are of excellent quality – deteriorates 'on dispense'. This reflects a mix of dispense parameters including product temperature, gas management, throughput and poor hygienic practices. Of these, temperature (too high or too low) and dispense gases (wrong mixture, product pick-up, pressure too high or too low) impact on brand presentation, dispense delivery and losses of product.

Hygiene and the associated microbiological risk are managed by regular and effective alkaline line cleaning to remove microbial biofilms *(2)*. In addition, keg couplers and taps *(2)* together with nozzles (7) should be cleaned and sanitised to minimize contamination of the system. Deteriorating dispense hygiene

results in the growth and metabolism of beer spoilage microorganisms which contribute to changes in beer flavour and aroma together with the appearance - *in extremis* - of haze and 'bits'.

Consumers 'drink as much with their eyes as with their mouth' *(8)* having expectations of appearance such as foam and clarity. Shifts in beer flavour and aroma due to the formation of compounds such as diacetyl, esters and acetic acid are viewed by consumers as being 'different', 'off' or 'wrong'. Whatever the interpretation or understanding, some (but not all) consumers will 'vote with their feet', leave the outlet, tell their friends or blame the brand and move to a different beer. Indeed, it is reported that 'nearly 95% of consumers would stop using a pub if beer quality was constantly poor'. Conversely from the same report, 'for nearly 90% of consumers, beer quality is essential or very important when selecting a venue' *(9)*.

Intuitively, draught beer of excellent quality 'sells'. Anecdotal reports suggest beer of high-quality leads to an uplift in sales (reportedly 10%) over beer of indifferent to poor quality. Hard evidence though is limited. One report *(3)* from a UK retailer links the frequency of line cleaning with the commercial performance of licensed premises. In terms of volume growth, cleaning every two weeks results in marginal (0.1%) growth with weekly (UK best

practice) cleaning resulting in almost 2% uplift. Conversely frequencies of between two and four weeks lead to about a 2% loss of volume. Despite the financial benefit, take up of best practice is patchy with an estimated third of UK draught beer dispensed through dirty lines *(9)*.

Assessment of draught beer quality in the UK on-trade has mostly focused on cask beer and is essentially qualitative. Cask Marque *(10)* provides an independent, empirical assessment of cask ale quality based on Assessors visiting subscribing accounts at least twice a year. Visits are unannounced and involve a yes/no measurement of temperature, clarity and, a sip test, to assess flavour and aroma. Accreditation to Cask Marque is communicated to consumers via a plaque or can be searched online via the CaskFinder app.

A recent review *(2),* reported that the brewing literature on draught beer quality is slight, with only 12 or so publications since the 1950's. However, reflecting the global reach of draught beer, these publications are from the UK, Finland, Germany, USA and Spain. Most studies on draught beer quality have used microbiological plate tests although ATP bioluminescence has also found application more recently.

Measurement of microbial loading does not easily relate to the consumer experience or to beer quality. The few studies

(2,11,12,13) have reported a range of values for commercial draught beer ranging in quality from 'good' (< 1000 colony forming units (cfu) per millilitre of beer) to 'unacceptable' (> 50,000 cfu/ml). These figures are aligned to the recommendations of the Deutsches Institut für Normung (DIN) 6650 standard 'dispense systems for draught beverages' *(14)*. Part 6 of this German standard covers 'requirements for cleaning and disinfection' and provides guidelines for the extent of microbial loading. Here 'a typical guideline value for a positive result with respect to microbial contamination would be 1000 cfu/ml, a value of more than 50,000 cfu/ml being considered unacceptable. If the count is 10,000 or higher, cleaning is necessary'. No guidance is given as to testing methodology, as the standard is generic for draught beverages including beer, wine, water, carbonates etc.

In this work and for the first time, draught beer quality has been quantified by 'forcing' samples post dispense. Such accelerated shelf life testing by storage at elevated temperatures has long been used in the brewing industry to assess the microbiological stability of beer in process and more recently to assess the hygiene of dispense tap nozzles *(2)*. 'Forcing' was first developed by Horace Brown *(15)* in the early 1870's to predict the spoilage of Burton ales brewed between October and May for sale in the Summer when, in the absence of refrigeration, there was no brewing. Samples of beer could be stored 'under such conditions of temperature as

would hasten the development of any of the adverse bacterial changes to which the beer was liable when stored under the ordinary conditions which rule in practice' *(15)*. The method involved newly racked beer being stored at (24-29°C) for between 10 days and three weeks. After forcing, beers were examined for flavour, clarity, present gravity, acidity and microscopically *(16)*. As noted by Kulka *(17),* the environment during forcing with a mixed microflora gradually changes. Accordingly, the beer during forcing becomes a 'better medium for growth, allowing development of some organisms at the end of the forcing period which were initially incapable of growth' *(17)*.

Here, forcing of draught beer samples was performed by incubating samples post dispense statically at 30°C for 4 days. The clarity of the samples - before and after incubation - was determined by measurement of absorbance.

3.2 Materials and methods

Microbiological media were obtained from Oxoid and cycloheximide (0.1%, w/v) from Sigma Aldrich.

Beer samples (250 ml) post dispense were purchased from on-trade licensed premises (pubs and bars) in Burton-on-Trent, Derby, Loughborough, Market Harborough and nearby villages. Beers were UK-wide keg brands including lagers (two categories of abv, ≤ 4.1% abv and > 4.1% abv), keg ale (≤ 4.1% abv and > 4.1% abv)

and stout (4.2% abv). Other products included nationally available and local cask-conditioned beers, wheat beers, local craft/microbrewery beers and keg cider (abv ≤ 4.1% abv and > 4.1% abv). Table S1 (in the on-line supporting information) provides an overview of the licensed premises and the beers that were sampled as part of method development. Post sampling, all activities were performed aseptically.

3.2.1 Method for draught beer quality

The method was used to assess keg beers (lagers, ales and stouts), keg ciders, cask beers and 'craft' unfiltered and unfined keg beers. On-trade samples were transferred *ex* tap or from glassware to sterile 250 ml Duran bottles. Samples were kept cold in transit and either processed on the day or stored overnight (4-6°C) before processing. After thorough mixing, 2 x 25 ml was transferred to plastic Universal bottles, the cap located on top (but not tightened – to allow gas transfer) and incubated statically at 30°C. Cycloheximide (4mg/L) was added to cask, unfiltered and unfined beers to suppress the growth of primary *Saccharomyces* yeasts.

Forced samples were thoroughly mixed by inversion – recalcitrant sediments were resuspended with a sterile plastic loop and well mixed. The absorbance of the samples was measured in duplicate at 660 nm (Jenway spectrophotometer 7315) in duplicate (1 ml) at the beginning of incubation and after *ca*. 96 hours at 30°C.

The absorbance of the sample was proportional to the degree of light scattering by suspended particles (yeast and bacterial cells, flocs, flakes etc.) The chosen wavelength has long been used to quantify yeast cultures *(18)*. Heavily contaminated dark beers were diluted (1:1) with water prior to measurement of absorbance.

There are numerous practical and operational unknowns in the on-trade that impact on draught beer quality. Accordingly, it is recommended that sampling of draught beers in a licensed account is performed more than once. To avoid 'first runnings' - which typically have a higher microbial load - samples were taken during busy trading sessions and not on opening.

3.2.2 Quality bands

The 'quality' of the beer post dispense was determined from the difference in absorbance of the two samples measured the beginning of incubation and after *ca*. 96 hours at 30°C. The increase in absorbance was used to classify the samples into four bands: A (0-0.3), B (> 0.3-0.6), C (> 0.6-0.9) and D (> 0.9) (Figure 3.1). The change in turbidity reflected the microbiological 'quality' at dispense, such that the A category ('excellent') with relatively little change in absorbance was superior to B ('acceptable') and which in turn was better than C ('poor') with D being of 'unacceptable' quality.

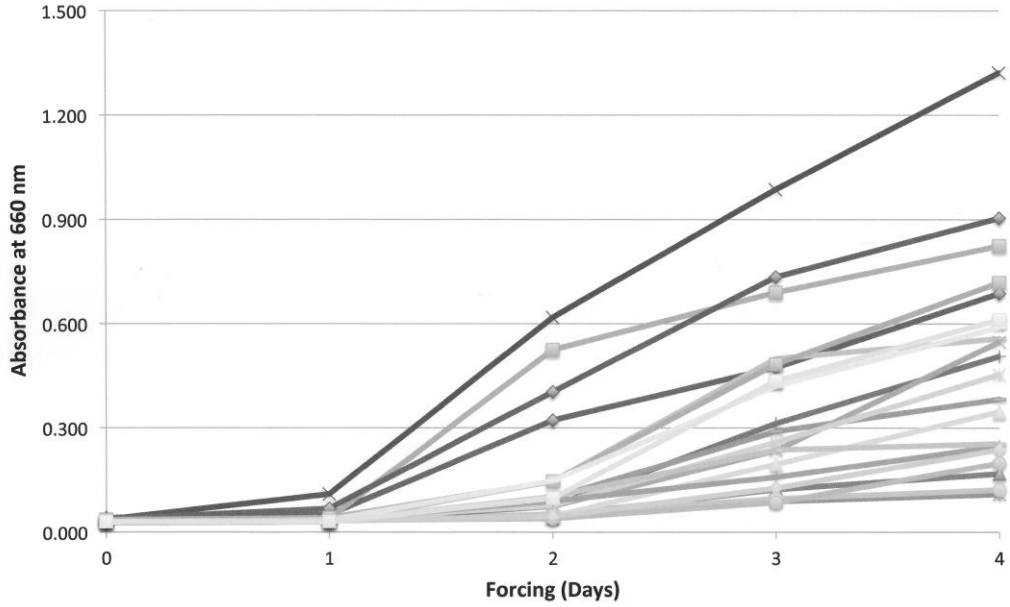


Figure 3.1: Changes in absorbance of draught beer samples on incubation at 30°C.

3.2.3 Quality index

For groups of samples (e.g. sampling all the taps on the bar), a 'quality index' was calculated from the sum of the individual quality bands (where A = 4, B = 3, C = 2, D = 1) divided by (number of samples x 4) x 100. If all samples are measured as excellent/quality band A, the quality index is 100%.

3.2.4 Microbiology

Samples post dispense from 24 public houses were diluted 10^{-1} and 10^{-2} and post forcing 10^{-4} and 10^{-5}. For each dilution, 0.1 ml (in duplicate) were spread onto selective agars. Raka Ray (with cycloheximide, 10 mg/L) plates were incubated anaerobically

(Oxoid Anaerogen) for five days at 30°C and WLN (Wallerstein Laboratory Nutrient) incubated aerobically for two days at 25°C.

3.3 Results and discussion

3.3.1 Forcing in Universal bottles

Disposable Universal bottles are of a standard shape (9 x 2.5 cm, with a conical base) and volume (30 ml), sourced in either polystyrene or polypropylene with plastic screw caps and or with a proprietary 'flow seal' cap to provide 'excellent sample containment'.

Beer spoilage assessed through 'forcing' depends on the mix of the spoilage microbiome and sufficient nutrients in the beer to the support growth of contaminating microorganisms. The availability of oxygen supports the growth of aerobic microorganisms in the microflora. However, under conditions of static incubation, transfer of air into the forced beer would be modest. The use of Universals with a 'flow seal' cap resulted in a lower value for draught beer forcing compared to the same samples processed with a loosely positioned standard screwcap.

To explore this further, draught samples - stout and lager (> 4.1% abv) - from the same outlet were forced in triplicate in Universals made of different materials (glass, polypropylene and three sources of polystyrene, including the flow seal cap) with the cap either tight and loosely positioned. The mean OD_{660} difference for

the stout was 1.405 (open) compared to 0.933 (closed) whereas for the premium lager was 1.006 (open) and 0.471 (closed). The Student's T-test (two tailed) showed the results between open and closed lids were significantly different at the $P < 0.001$ level confirming the need for the caps to be loosely positioned on the Universal.

3.3.2 Reproducibility

Whilst 'forcing' is a long-established method, its reproducibility in the context of draught beer quality needed to be validated. In all, 12 keg beers (7 x lager (abv ≤ 4.1%), 2 x lager (> 4.1% abv), 3 x ale (abv ≤ 4.1%)) from five public houses were assessed in quintuplicate. Table 3.1 reports the mean (± standard error) of the absorbance of individual samples post forcing at 30°C for four days. The reproducibility of the method is clear, with a consistent quality band for the five replicates of the 12 different samples of forced draught beer.

Table 3.1 Reproducibility of the forcing method.

On-premise	Beer	Mean A_{660}	± sem	Quality band
B1	Lager (≤ 4.1% abv) (SL 3)	0.120	0.012	5 x A
	Lager (≤ 4.1% abv) (SL 6)	0.535	0.008	5 x B
B2	Lager (≤ 4.1% abv) (SL 3)	0.146	0.028	5 x A
	Ale (≤ 4.1% abv) (SKA 5)	0.517	0.012	5 x B
B6	Lager (≤ 4.1% abv) (SL 3)	0.419	0.026	5 x B
	Lager (≤ 4.1% abv) (SL 4)	0.314	0.005	5 x A
B8	Lager (>4.1% abv) (PL 1)	0.810	0.010	5 x C
	Lager (>4.1% abv) (PL 5)	0.091	0.005	5 x A
	Lager (≤ 4.1% abv) (SL 3)	0.154	0.006	5 x A
	Ale (≤ 4.1% abv) (SKA 5)	0.713	0.014	5 x B
B9	Lager (≤ 4.1% abv) (SL 3)	0.073	0.032	5 x A
	Ale (≤ 4.1% abv) (SKA 1)	0.680	0.026	5 x B

- Public houses were sampled in Burton-on-Trent (B). Keg beers are described as lager (≤ 4.1% abv) (SL), lager (>4.1 abv) (PL) and ale (≤ 4.1% abv) (SKA).
- An overview of the brands and licensed premises can be found in Table S1 (Appendix I).

3.3.3 Incubation – static v mixed

Initially, the impact of mixing was assessed with 22 keg lagers (abv ≤ 4.1%) and 12 keg ales (abv ≤ 4.1%) from 16 public houses. Samples were forced for four days either statically or with daily inversion. A more detailed experiment was then performed with 11 draught lagers (abv ≤ 4.1%) and nine draught ales (abv ≤ 4.1%) from 10 public houses. The change in turbidity was assessed after four days with daily mixing and statically after four, five, six and eight days. A Student's T-test (two tailed) showed no significant difference between daily mixing and static incubation (P = 0.97).

3.3.4 Incubation – impact of time

Further work (Figure 3.2) suggests the impact of mixing though is marginal with a small shift in the mixed samples from quality band A to B compared to static. Importantly, the C and D quality bands were the same with or without mixing. However, extending the time of static incubation from four up to a maximum of eight days had a marked impact. As might be anticipated, increasing the time of incubation reduced the number of 'excellent' (A band) samples and progressively increased the 'poor' (C) and 'unacceptable' (D) categories. This is reflected by calculation of the quality index which declined with time from 80% after four days (81% static, 79% daily mixing) to 71% (five days), 70% (six days) and 64% (eight days).

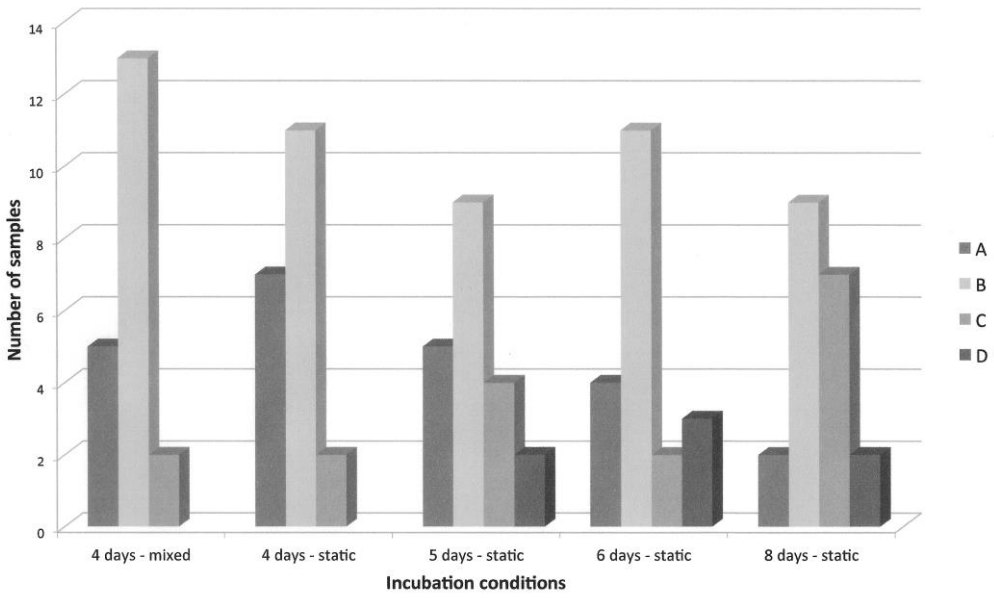


Figure 3.2: Impact of mixing and incubation time of draught beer on the forcing test.

3.3.5 Processing and overnight storage

Processing of trade samples on the same day as they were sampled was not always possible. On such occasions, bulk samples were stored overnight at 4°C. It would be anticipated that such a treatment would not impact significantly on the outcome of the forcing test. To confirm this (or not) the forcing of ten beers (and a cider) were compared with and without storage, 17 of the 20 results were unchanged with three changing by one quality band (two up and one down). A Student's T-test (two tailed) showed no significant difference between samples processed on the same day as sampling or after overnight cold storage (P = 0.53).

3.3.6 Suppressing the growth of brewing yeasts

The forcing test predicts quality by amplifying the indigenous yeasts and bacteria present in the beer post dispense. Unlike filtered keg products, cask and unfiltered/unfined draught beers will contain primary brewing yeasts because of the beer style and not because of poor dispense hygiene. Clearly, the presence of brewing yeasts can grow in a forcing test and distort the measurement of draught beer quality. To suppress the growth of primary yeasts, cycloheximide (*aka* actidione) was added prior to forcing. This antibiotic has long been used in microbiological media to suppress the growth of brewing yeasts whilst allowing the growth of 'wild' yeasts and bacteria *(19)*. As ever with brewing microbiology, things are not black and white. The inclusion of cycloheximide *(20)* can suppress the growth of *Saccharomyces* wild yeast (e.g. *S. diastaticus*) which have been reported to be 'prolific beer spoilage microorganisms' responsible for trade returns of draught beer *(21)*. However, the addition of cycloheximide does allow the growth of non-*Saccharomyces* contaminants *(20)* such as *Brettanomyces, Pichia, Candida and Hansenula,* which have been reported in draught beer *(22,23,24)*.

Analysis of cask beers, wheat and unfiltered beers from four public houses with and without the addition of cycloheximide (4 mg/L) suggested that the inclusion of the inhibitor resulted in marginally more spoilage, but this was dependent on the public house.

Indeed, a Student's T-test (two-tailed) showed that addition of cycloheximide did result in a significant difference at the $P \leq 0.05$ level.

This is not surprising and reflects the likely diversity and mix of the spoilage microbiome within and between licensed premises. However, despite these complexities, it is suggested that the addition of cycloheximide should be routinely added to samples of cask, unfiltered and unfined beers post dispense and prior to using the forcing test.

3.3.7 Microbial loading post dispense and DIN 6650-6

The DIN standard recommendations for the microbiological loading of beverages (in this case beer), provides a framework for the assessment of post dispense quality. Four categories of loading are detailed in the standard (i) < 1000 cfu/mL, (ii) 1000-10,000 cfu/ml, (iii) 10,000-50,000 cfu/ml and (iv) > 50,000 cfu/ml. Table 3.2 reports the four categories, in terms of aerobic and anaerobic selective agars for the work reported here. Here, an average of 32.5% of the samples were in the 'positive result' category with 32% in the 'acceptable' category. However, 18% were in the 'cleaning is necessary' band, with 17.5% 'unacceptable'. The highest microbial count of the samples reported here (in the > 50,000 cfu/ml category), was 300,000 cfu/ml although up to 1,000,000

cfu/ml have been reported in draught beer samples in Germany *(11)* and Finland *(12)*.

Although microbiological loading is the metric that the DIN standard uses to assess the quality of draught beverages, there are no recommendations regarding media or incubation conditions. Accordingly, as noted above, there are different interpretations of draught beer 'quality' in the trade depending on whether the media is – for example - WLN incubated aerobically or Raka Ray incubated anaerobically. The major draught beer spoilage organisms *(2,12)* have a mixed response to the presence of oxygen, and include the aerotolerant anaerobic bacteria (*Lactobacillus*, *Pediococcus*), aerobic bacteria (*Acetobacter*, *Gluconobacter*), facultatively aerobic yeasts (*Saccharomyces*, *Brettanomyces*) and aerobic yeasts (*Pichia*, *Candida*). However, which predominate to spoil draught beer reflects the microbial loading/mix together with the beer composition (nutrients, iso-alpha acids, pH etc.), the concentration of carbon dioxide and availability of dissolved oxygen. In terms of processing, keg beer is assumed to be effectively anaerobic whilst cask beer can pick up oxygen as the container is dispensed. Despite this, container couplers and connectors together with taps and nozzles are hot spots for contamination *(2)* and are aerobic environments.

3.3.8 Microbial loading post dispense v forcing

The microbiological loading and the forcing test are quantified by the measurement of cell numbers. Both approaches require a period of incubation of two to seven days (microbiology) or four days (forcing). The methods differ, in that plate counts reflect the viable organisms detectable on selective agars on sampling, whereas the forcing approach amplifies the number of yeast and bacteria able to grow in the beer ex dispense. Despite these differences, the DIN microbiological approach and the forcing test, both categorise beer quality through microbiological loading (directly or indirectly) from low (good quality) to high (bad quality).

In addition to reporting the DIN classification for trade samples, Table 3.2 also the same trade samples assessed using the forcing test. Of the 52 samples, Table 3.2 shows that 35% of the population were in quality band A, 44% in B, 17% in C and 4% in D. Subjectively, 4% of the samples being assessed as 'unacceptable' is a more realistic measure than the 18% flagged by the DIN approach. Linear regression analysis of the two approaches shows the best correlation (R^2 = 0.8737, y=2.0024x-25.059) with the combined results (aerobes and anaerobes) from the DIN categories against that of forcing. Despite this, the relationship is skewed such that the DIN approach of microbiological loading underestimates quality bands A (excellent) and B (acceptable) but overestimates bands C (poor) and D (unacceptable). A likely explanation is that

microbiological testing quantifies a mix of microorganisms some of which are 'environmental' and accordingly are unable to spoil beer.

Table 3.2 Microbial loading vs forcing

Microbiology					Forcing		
cfu/mL	DIN Description*	% Aerobes	% Anaerobes	% Combined (%Aerobes +%Anaerobes/2)	Band	Description	%
< 1000	Positive	25	40	32.5	A	Excellent	35
1-10,000	Acceptable	35	29	32	B	Acceptable	44
10-50,000	Cleaning required	19	17	18	C	Poor	17
> 50,000	Unacceptable	21	14	17.5	D	Unacceptable	4
	Total	100	100	100		Total	100

- Samples (52) post dispense were taken from 24 licensed premises and represent 15 different brands of keg lager, ale and cider together with cask ale (details are reported in Table S1 in the on-line supporting information).
- The DIN standard 6650-6 defines the microbial counts (as cfu/ml) (i) < 1000 as a 'positive result', (ii) 1-10,000 as 'acceptable', (iii) 10-50,000 'cleaning is necessary' and (iv) > 50,000 as 'unacceptable'.
- % Aerobes and % Anaerobes = % of samples that had aerobic/anaerobic count within each band of DIN standard. % Combined = mean % of samples within each DIN band.
- 'Aerobes', 'anaerobes' and 'forcing' are as defined in the Materials and Methods.

3.3.9 Microbial loading post dispense v forcing – an explanation

Conventional 'traditional' microbiological testing has its limitations. One limitation is that there is no universal microbiological medium, so to build a picture, different agars are used to 'select' for different microorganisms. In this work, WLN is selective for aerobes (yeast and acetic acid bacteria) whereas Raka Ray is used to quantify anaerobes (lactic acid bacteria). However, this is complicated by being unable to confidently extrapolate growth on a plate to the spoilage of beer. Microbiological testing is directional but offers no guarantees of robustness and accuracy. This is further compromised by considerations which contribute to microorganisms being unable to grow on selective agars. This can be due to poor growth rate, the recovery and growth of nutritionally fastidious microorganisms, viable but non-culturable organisms such as *Lactobacillus* species *(25)* and the loss of support (e.g. trading nutrients, quorum sensing etc) from the microbiome of the sample. Accordingly, quantification of microorganisms on agar plates does not necessarily mean those organisms can grow in/spoil beer *in situ*. Indeed, these results challenge the relationship between conventional microbiological analysis in breweries and the relevance of such analysis to possible spoilage.

The forcing test quantifies the increase in absorbance due to the growth of microorganisms in beer. This may be compounded by the cell size and shape of spoilage microorganisms that impact on light scattering and therefore absorbance. Although spoilage is (invariably) from a consortium of diverse yeasts and bacteria, there will be occasions where sample turbidity is skewed by the mix of large (yeast) and small (bacterial) cells.

3.3.10 Application of the method

Whilst 'best practice' in draught beer dispense is increasingly defined and communicated *(2),* measurement of quality has attracted little attention. Indirect measurement of poor quality through 'losses' can be commercially relevant and can result in the loss of business and ultimately closure. The method reported here is simple (although taking four days) but provides real differentiation of beer quality based on forcing the indigenous microorganisms present at the point of dispense. Accordingly, this method provides a tool to assess beer quality in the on-trade/on-premise against a variety of parameters both routine and in response to changing practices. Obvious comparisons include (i) public houses within retailer groups, (ii) brands within and between public houses and (iii) the impact of outlet factors. Beyond the routines, the method will add value in assessing and validating the impact of innovation such as (i) line cleaning solutions, (ii) line

cleaning frequency and technologies, (iii) dispense line composition (including FOB detectors) and (iv) end to end cooling of beer from keg to tap. The method would also lend itself to a quantifiable (rather than qualitative yes/no) assessment of beer quality in the on-trade licensed premises. Handled appropriately this method could add real value to consumers and their understanding of the importance of beer quality.

3.3.11 Validation of the method

The method reported here has been successfully used to assess the quality of draught beer in > 65 public houses and > 500 samples of beer.

3.4 Conclusions

Quantitative assessment of draught beer quality that relates to the consumer experience has not been reported. The method provides a direct assessment of the microbiological status of beer at the point of dispense which is then subsequently amplified by forcing. Beer of excellent quality contains low numbers of beer spoilage organisms which on incubation at 30°C develop little turbidity (A_{660} < 0.3, quality band A). Conversely forcing beer of poor microbiological quality results in high turbidity with A_{660} > 0.9 (quality band D).

The method does not identify the source of contaminating microorganisms but reflects the total dispense system.

Accordingly, there is no insight into potential hotspots of contamination although the dispense line, fob detector and nozzles are typically the primary candidates *(2)*. In addition, the beer itself and, possibly, glassware may also contribute to the microbial mix that presents on forcing.

The forcing method presents a different picture to the measurement of microorganisms on agar plates. It is proposed, that despite the advocacy of the DIN standard, microbiological testing is an unsatisfactory approach to describe draught beer quality. This reflects complex factors that may – on the one hand - exaggerate the microbial loading through the use of selective microbiological media or – on the other - underestimate viable but non-culturable organisms. The forcing method described here quantifies those microorganisms in draught beer that can grow and spoil beer. Accordingly, the forcing test is recommended as a simple method to quantity draught beer quality.

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4 A SURVEY OF ON-TRADE BEER QUALITY

Foreword

After development of the method (see Chapter 3), the second phase of my PhD was to conduct a survey of public houses for on-trade beer quality.

The following manuscript presented in this chapter was first published in the Journal of the Institute of Brewing on the 10th December 2018.

My contribution to this paper consisted of being lead author and producer of the data presented here, under the supervision of Dr David Quain.

Draught beer hygiene: a survey of on-trade quality

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Abstract

The quality of draught beer in 57 on-trade licensed premises in 10 locations in the UK Midlands was assessed using a forcing test. Of 149 samples of standard lager ('SL', abv ≤ 4.2%), 44% were in the 'excellent' quality band compared to 16% of 88 samples of keg ale ('KA', abv ≤ 4.2%). Of the total of 237 samples, > 90% were represented by two lager and two ale national brands. There were differences in the quality index (QI) between the brands, with lager SL3 having a QI of 84% compared to 72% for lager SL6, 71% for ale KA5 and 68% for ale KA1. The susceptibility of the four brands to spoilage was assessed using a challenge test with microorganisms taken from forced draught samples of the brands. Ale KA5 (challenge test QI = 87.5%) was the most resistant to spoilage followed by lager SL3 (81.3%), lager SL6 (75%) and ale KA1 (62.5%). Keg beers in accounts with a national cask beer quality

accreditation had the same QI as those without accreditation. Analysis of price versus quality showed that the most expensive price band had the lowest quality. Draught beer quality declined as the number of dispense taps increased across the bar. It was also noted that dispense of beer into branded half pint glasses had variable take-up, with lager SL3 served in the correct branded glassware on 71% of occasions but with only 5% of occasions for lager SL6. None of the keg ales were served in correctly branded glassware.

Keywords: dispense, quality, survey, spoilage

Short title: Survey of on-trade beer quality

4.1 Introduction

Between 2000 and 2016, UK beer sales declined by 12.9 mhL from 56.6 to 43.7 mhL *(1)*. Over the same period, draught beer sales fell by 15.6 mhL from 35.2 to 19.6 mhL. This reflects the decline in draught beer from 62.3% of total sales in 2000 to 44.7% in 2016. In terms of the category, the UK draught market in 2016 was dominated by pasteurised keg beers including lager (62.4%), keg ale (12.8%) and stout (6.7%). Unpasteurised cask beers account for 18.1% of the draught beer market.

There are a host of reasons for the long-term decline in beer sales in general and draught beer in particular. A PEST (political, economic, social and technological) analysis of the on-trade

identified some 27 factors which – to a lesser or greater extent - are considered to have contributed to the decline *(2)*. Of these, 'quality' is a perennial issue for draught beer. Quality can be compromised by the dispense process (slow throughput, temperature, over/under carbonation), glassware (dirty, wrong glass) and hygiene (bacteria and yeast generating off-flavours, aromas and haze). Although draught beer hygienic quality varies widely, measurement ranges from indirect (data logging) *(3,4)*, subjective (clarity, aroma, appearance) *(5)* to objective (isolation and quantification of microorganisms) *(6-8)*. As these approaches have their limitations, a new approach has been reported to assess the hygienic quality of draught beer based on the microbial loading at dispense *(8)*.

The method *(8)* is based on the long established 'forcing' principle, in this case, forcing draught beer at 30°C for four days. The increase in turbidity of beer post incubation relates to the initial loading of beer spoilage microorganisms. Accordingly, beer of 'excellent' quality exhibits little or no increase in turbidity, which with increasing initial microbial loading reduces to 'acceptable' through to 'poor' or, worse still, with a major increase in turbidity, 'unacceptable'.

Here, we report the application of the forcing method to assess the quality of draught beer in the on-trade. Samples (237) of leading

brand keg ales and lagers were purchased in 57 on-trade licensed premises ('accounts') in 10 locations on typically two occasions. In addition to beer quality, other metrics were recorded including quality accreditation, cost, number of taps on the bar and the use of branded glassware.

4.2 Materials and methods

Beer samples (half pint) were purchased locally from on-trade licensed premises (pubs and bars) in two cities (Derby, Nottingham), three towns (Burton-on-Trent, Loughborough, Market Harborough) and five villages (near to Derby). The combined population of these locations is about 750,000. For clarity, a city is defined as having a cathedral and/or a university, a town has a market and a village has a church.

The work reported here was over a period of nine months from May 2016 to January 2017. The focus of the survey was on two draught beer categories, 'standard' lager (SL) and keg ale (KA). Both categories have an abv of ≤ 4.2% *(1)* and are flash pasteurised into keg. In all, 237 samples were purchased, comprising of 149 lagers and 88 keg ales. For both categories, two brands predominated accounting for 96% (lager SL3 and SL6 - both 4% abv) and 93% (ale KA1, KA5 - both 3.6% abv) of the samples. In accounts without a keg ale, a second standard lager sample (≤ 4.2%) was purchased.

4.2.1 Sampling

Of the 57 accounts, 44 were visited twice, 11 were sampled more than twice and two were sampled once. Sampling was between 13.00 – 18.30 on various days of the working week (Monday to Friday) and was covert. Repeat sampling was to reduce the influence of unknown factors that impact on draught beer quality, such as line cleaning, throughput, length of time on-sale etc. Samples were decanted directly from the half pint glass into sterile Duran (250 mL) bottles. Some pick up of oxygen was inevitable but is unlikely to impact on forcing as draught keg beer has been found to contain oxygen at the point of dispense (unpublished observations). Samples were stored in a cool box with cold blocks to minimise warming during transit.

4.2.2 Forcing test

Draught beer quality was determined using a forcing test *(8)*. Samples were processed on the same day as sampling or, where required, next day after overnight storage in a refrigerator. Samples (2 x 25mL) in sterile polystyrene universal bottles were incubated at 30°C for 96 hours. Quality was measured by the increase in absorbance at 660 nm. Beers were classified as excellent or band A (increase in absorbance between 0-0.3), acceptable/band B (>0.3-0.6), poor (C, >0.6-0.9) and unacceptable (D, > 0.9).

4.2.3 Quality index

For groups of related samples, a 'quality index' was calculated from the sum of the individual scores for each quality band (where A = 4, B = 3, C = 2, D = 1) divided by (number of samples x 4) x 100.

Quality index (%) = $\frac{\Sigma\ quality\ score}{number\ of\ samples\ x\ 4}$ x 100

If all samples are measured as excellent (quality band A), the quality index is 100% whereas if all samples are in quality band B (acceptable) the index is 75%

4.2.4 Challenge test

The vulnerability to spoilage of the lager and ale brands (SL3, SL6, KA1 and KA5) sampled in this work was compared in challenge tests. Draught beer samples of each brand from four different accounts were forced (as above). An aliquot of hazy beer equivalent to A_{660} = 1 was diluted with sterile water to a final volume of 5 mL. From this, 0.1 mL (A_{660} = 0.02) of brand specific spoilage microorganisms were inoculated into all four brands (25 mL, from pasteurised cans or bottles) in triplicate, forced at 30°C for 96 hours and the increase in A_{660} measured.

4.2.5 Data collection

The work reported here was observational and did not interfere in its generation *(9)*. Accordingly, sampling was random with no

influence on handling or storage, staff training or hygienic status of the dispense systems.

4.3 Results and discussion

4.3.1 Quality of draught ale and lager – all accounts

Analysis of the quality of 237 samples of draught beer from 57 accounts (Figure 4.1) showed clear differences between lager and ale. Of 149 samples of draught lager, 44.3% were in the 'excellent' quality band compared to 15.9% of the 88 ale samples. Despite this, approximately three quarters of the lager (77.9%) and the ale samples (72.7%) were either 'excellent' or 'acceptable'. Overall the quality index of the lager samples was 79.7% compared to 71.0% for the ale samples.

4.3.2 Quality of draught ale and lager – common accounts

In 42 on-trade licensed premises, both standard lager and keg ale were sampled. The quality of 90 lager samples was compared with 87 keg ale samples. Overall, the draught lager samples had a quality index of 80% compared to 71.3% for draught ale. In terms of the 'excellent' quality band there was a marked difference with 39 (42.4%) samples of lager rated as excellent compared with 12 (13.3%) of the ale samples.

A Student's t-test (two-tailed) confirmed that there was a significant difference between the two data sets, with a P-value of

0.002 showing that lager was of significantly better quality than keg ale in these accounts.

4.3.3 Quality of two draught lager brands (abv 4%)

Of the 149 samples of draught standard lager, 142 were from two of the leading UK on-trade brands, SL3 and SL6. Analysis (Table 4.1) using the forcing test, showed lager SL3 (109 samples) to be of better quality with 52.3% 'excellent' (A band) compared with 23.5% of lager SL6 (33 samples). At the other end of the quality spectrum, none of the SL3 lager samples were 'unacceptable' (D band) compared to 5.9% of SL6 samples. Overall these differences are reflected by a quality index of 84.0% for lager SL3 compared to 72% for lager SL6.

High street or main shopping street accounts with 20 and often more taps are able to offer the same brands across the bar alongside similar competing brands. To minimise the account to account 'noise' in comparing lagers SL3 and SL6, 18 samples of each brand were obtained from 12 accounts. Two-way ANOVA, with account as the random factor and brand as the fixed factor, showed the brands to be statistically significantly different ($P<0.0001$).

4.3.4 Quality of two draught ale brands (abv 3.6%)

Keg ale is in long-term decline, accounting for 9.8% (mixed gas of carbon dioxide and nitrogen) and 2.9% (CO_2) of draught beer

sales in the UK in 2016 *(1)*. However, the keg ale category is still offered in many accounts although usually just one brand. Two of the leading UK on-trade (mixed gas) ale brands – KA1 and KA5 - were sampled and analysed (82 samples) using the forcing test (Table 4.1). As might be anticipated from the results for the ale category (above), the predominant quality band for both brands was 'acceptable' with 58.7% (KA1) and 42.1% (KA5). However, 27% (KA1) and 36.8% (KA5) of the samples are in the 'poor' (band C) category. This was reflected by the quality index of *ca.* 70% for both brands (Table 4.1).

4.3.5 Quality in accounts

The quality index for the beers sampled in the 57 accounts varied widely (Figure 4.2). The average number of samples from an account was 4.2 ranging from two to a maximum of eight. The quality index for broadly half of the accounts were in the acceptable to excellent bands (75-100%). Conversely 50% were dispensing beer in the poor category (50%-74%).

2907 **Table 4.1** Quality of draught beer.

	Lager SL3			Lager SL6			Ale KA1			Ale KA5		
Quality band	No	%	QI (%)	No	%	QI (%)	No	%	QI (%)	No	%	QI (%)
A	57	52.3	84.0	8	23.5	72.0	6	9.5	68.3	4	21.1	71.1
B	34	31.2		15	44.1		37	58.7		8	42.1	
C	18	16.5		8	23.5		17	27		7	36.8	
D	0	0		2	5.9		3	4.8		0	0	
total	109			33			63			19		

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4.3.6 Spoilage

Generically, beer is inhospitable to microorganisms, with numerous compositional hurdles including low pH, ethanol, colour, hop bitter acids, reduced nutrients (e.g. free amino nitrogen), low oxygen, low temperature, undissociated sulphur dioxide *(10,11)* and phenolic compounds *(12)*.

Accordingly, for spoilage, there is selective pressure for environmental organisms that can survive and, critically, grow in draught beer. These include bacteria (*Lactobacillus, Pediococcus*, *Acetobacter)* and yeasts (*Saccharomyces, Brettanomyces, Pichia and Candida*) *(7)*. Spoilage of draught beer is ill-defined and reflects the mix of contaminating microorganisms. Outcomes of spoilage include acidification, super attenuation, sourness, haze and a blend of aromas (and flavours) from esters, higher alcohols, phenols, organic acids, diacetyl, short chain fatty acids and sulphur compounds *(7)*.

Most studies of beer spoilage have focussed on hop-resistant *Lactobacillus* and *Pediococcus*. This reflects the report that these genera account for 60–90% of microbiological spoilage events in Germany between 1980-2002 *(13)* and 2010-2013 *(14)*. Despite the generic hurdles, growth of hop resistant lactic acid bacteria (notably *L. brevis*) is supported by maltose, maltotriose and maltotetraose *(14)* together with organic acids including citrate,

pyruvate, malate and succinate *(11,14,15)*. Further, Rainbow *(16)* noted that beer spoilage lactobacilli need 'exogenous supplies of most α-amino acids, several growth factors of the vitamin B complex and one or more purine and pyrimidine bases.

For draught beer, the concentration of spoilage organisms in the dispense system is managed by the application of hygienic practices. Key to this is effective and regular line cleaning *(7)* which removes biofilm from surfaces (line, connectors, FOB detectors). The frequency of line cleaning is often compromised such that one in three pints is dispensed through an unclean beer line *(3,4)*. This inevitably damages beer quality and increases wastage. Further, managing the entry of yeast and bacteria at the 'ends' of the dispense system are recommended by sanitising the keg coupler/spear and tap/nozzle *(7,8,17)* but take up by the on-trade is at best poor. Product throughput also contributes to quality and ideally a keg should be consumed within a week which was only achieved in 39% of all keg taps in the UK, as reported in the 2017 beer quality survey *(4)*. In practice, time on sale varies widely within and between accounts reflecting brand popularity, container size, trading hours, footfall and consumer demographics. Of course, commercially, the bottom line is to empty the container irrespective of how long it is on sale.

In this work, using the forcing test, there were differences in draught beer quality/spoilage between brands and categories (ale and lager) (Table 4.1, Figure 4.1). Whilst, this may reflect 'account' factors (above), beer composition could also contribute as, in challenge tests, beers have been reported to vary in susceptibility to microbial spoilage *(10-12,18)*. Accordingly, the spoilage of the major brands in this work was compared by inoculating (spoiled forced) samples of draught beer into the parent brand and the other three brands with microorganisms from SL3 into SL3, SL6, KA1 and KA5, SL6 into SL6, SL3, KA1, KA5 and so on.

Using this standardised challenge test, the 'spoilability' of the four brands could be compared. Figure 4.2 shows that the four brands respond differently to draught beer spoilage organisms. Calculation of the quality index of the individual lager brands suggests that SL3 (81.3%) was slightly less susceptible to spoilage than SL6 (75%). However, keg ale KA5 (87.5%) was markedly more robust to spoilage than KA1 (62.5%). Comparison of the quality index of samples in trade (Table 4.1) with the quality index from challenge testing (above) shows, with the exception of KA5, a similar outcome with lager SL3 (84 v 81.3%), SL6 (72 v 75%), KA5 (71.1 v 87.5%) and KA1 (68.3 v 62.5%). For ale KA5, the quality index for the trade samples was derived from a comparatively small number of samples compared to the other brands.

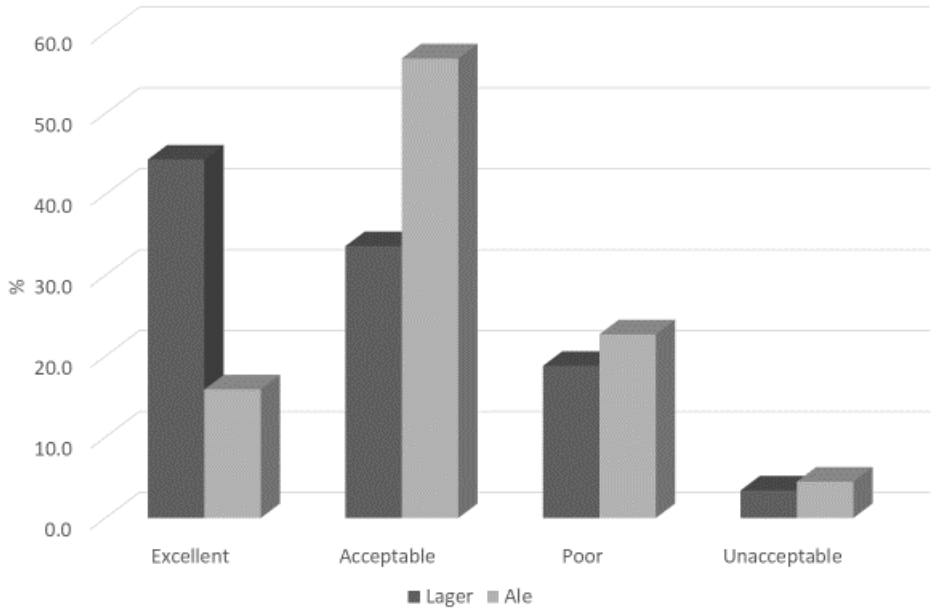


Figure 4.1: The quality of draught lager and ale in the on-trade.

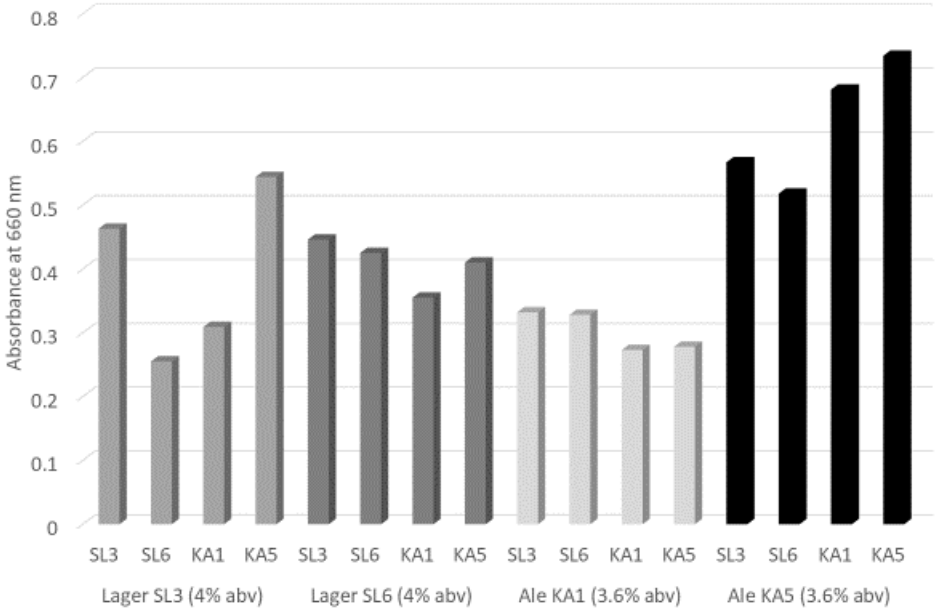


Figure 4.2: The susceptibility to spoilage of lager and ale brands.

4.3.7 Accreditation and quality

Assessment of draught beer quality in the UK on-trade has mostly focused on cask beer and – with the exception of temperature - is qualitative. Cask Marque *(5)* a non-profit making organization was

established in 1998 with the laudable aim to 'address the void in beer quality'. Its assessors visit subscribing accounts at least twice a year. Visits are unannounced and involve a yes/no measurement of temperature, clarity and, a sip test, to assess flavour and aroma of cask beers.

For the work reported here, it was noted whether the on-trade accounts were Cask Marque accredited. Of the 57 accounts sampled, 29 were Cask Marque accredited and 28 were not. Analysis of beer quality (Table 4.2) showed no difference in individual quality bands or overall beer quality in accounts with Cask Marque (QI = 76.8%) and those without (QI = 76.1%). As the focus of Cask Marque is on cask beer it is perhaps not surprising that keg beer quality was – in this study – indistinguishable from the quality of keg beer in accounts which are not accredited. However, a 'halo effect' might be anticipated where the 'quality message' underpinning the dispense of cask beer contributes to the assurance of keg beer quality.

Table 4.2 Quality of draught beer from accounts with and without quality accreditation.

Accreditation	No of Accounts	No of Samples	Quality band (%)				Quality index (%)
			A	B	C	D	
Cask Marque	29	126	34.1	42.9	19.0	4.0	76.8
None	28	111	33.3	41.4	21.6	3.6	76.1

4.3.8 Price verses quality

The linkage between price and quality has long been part of the marketing mix. McConnell *(19)* labelled the same commercial bottled beer at three different price points: high, medium and low. Using a cohort of 'sixty beer drinkers' they demonstrated that the higher priced brand was perceived to be of higher quality than the medium-priced brand. Building on this, Jacoby *et al., (20)* confirmed the linkage between price and perceived quality when it was the only signal available to consumers. However, branding had a greater impact on the perception of quality particularly 'for brands with strong positive images'. Reassuringly though, consumers were able to discriminate quality differences in the beers using 'only taste and aroma cues'.

The quality of the individual beers against the combined price of the samples from each on-trade account is reported in Table 4.3. The beers that were sampled were predominately national brands and, in the case of the lagers, supported by TV advertising. As the work reported here was covert, only the combined price of the two lager and ale samples was captured. In 2016, the average price in the UK for a pint of draught lager was £3.38 and for ale £2.99 *(1)*. This is equivalent to an average combined price of £3.19 which appropriately is in the most popular band (£3.01-3.50) accounting for 39% of the samples. Indeed, this price band had the best quality index of the four price bands being marginally better than the two cheaper bands. However, the most expensive price band (£3.31-4.00) had a notably lower quality index.

Table 4.3 Price of draught beer versus quality.

Price band (£)	Number of Samples	Quality band (%)				Quality Index (%)
		A	B	C	D	
2.01-2.50	54	35.2	42.6	18.5	3.7	77.3
2.51-3.00	63	30.2	50.8	15.9	3.2	77.0
3.01-3.50	92	38.0	41.3	17.4	3.3	78.5
3.51-4.00	28	25.0	25.0	42.9	7.1	67.0

4.3.9 Number of taps and location

In the UK, the number of dispense taps on the bar varies greatly. In this work, the accounts were defined by the number of taps from ≤10 to 11-20 and ≥21. Table 4.4 shows the distribution of the 57 accounts by village, town and city and number of taps. However, the draught beer quality index declined as the number of taps increased from ≤10 (88%) to 11-20 (76.7%) and ≥21 (75.9%). This decline in quality may reflect usage as it is likely that as the number of taps increase, some – typically away at either end of the bar - are only used during heavy trading sessions from Thursday to Sunday. Indeed, it has been reported that 16-20% of keg beer taps deliver less than 20 pints per week *(4)*. Such 'overfonting' will result in the quality of beer in underused taps being compromised. However, the number of taps per account increased with the size of the community (Table 4.5) such that half of the accounts sampled in towns and cities had > 21 taps compared to none of the village accounts. Price though was broadly comparable in the towns and cities but was higher in village accounts possibly reflecting the reduced competition in these locations.

3059 **Table 4.4** Number of draught beer taps versus quality.

Taps	Location	No of accounts	Samples	Price (£)	Quality band				Quality index (%)
					A	B	C	D	
≤10	Village	3	8	3.45	2	6	1	0	88.0
	Town	3	8	3.00	5	2	1	0	
	City	2	9	3.09	1	4	3	0	
11-20	Village	9	34	3.20	13	10	9	2	76.7
	Town	4	16	3.04	6	7	3	0	
	City	11	55	3.31	18	26	8	3	
≥21	Village	0	-	-	-	-	-	-	75.9
	Town	8	30	2.64	13	13	4	0	
	City	17	77	2.75	22	32	19	4	

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Table 4.5 Account location versus price of draught beer.

	No of taps	No of accounts	%	£
Village	≤10	12	25	3.25±0.09
	11-20		75	
	≥21			
Town	≤10	15	20	2.82±0.19
	11-20		26.7	
	≥21		53.3	
City	≤10	30	6.6	2.99±0.27
	11-20		36.7	
	≥21		56.7	

4.3.10 Glassware

Glassware has become an important part of the draught beer 'offer' in the UK. Although the unbranded 'conical' and 'nonic' glass are commonplace, for some accounts single (or multi-brand) glassware increasingly predominate. Branded glasses can be of different shapes *(21)* as this can contribute to the sensory experience *(22)*. Other marketing tools may include the thickness of the glass and tactile design when the glass is held *(23)*. Functionally, laser etched 'nucleation sites' on the base of (typically) lager glassware promote bubble release to replenish the foam. In practice, these interventions can be compromised by

dirty glassware – estimated at one in five glasses *(3)* – or dispense into the wrong branded glass.

Although not impacting on beer quality, the glassware that samples were dispensed into was recorded. It is apparent that the uptake and usage of branded glassware varies widely between independent, small and large pub groups. Although a small sample size, there were clear differences between the availability of branded half-pint glassware for standard lagers and keg ales. Lager SL3 was served in the correct branded glassware on 71.4% of occasions whereas the frequency was only 5% for lager SL6. Indeed, lager SL6 was served in SL3 glassware in 20% of the accounts the brand was sampled.

None of the keg ales were served in correctly branded glassware, indeed 13% of samples were served in the wrong branded glass with the majority (87%) in unbranded glasses. The lack of (half pint) glassware is not unexpected as the focus is on branded pint glasses but also may reflect the declining market share of the keg ale category.

4.3.11 Insights – relevance and considerations

This survey of draught beer suggests that quality varies widely from excellent through to unacceptable and that the quality of keg lager is superior to keg ale. It is suggested that there is nothing unusual about dispense practices, configuration or complexity in

the Midlands and that these results are relevant to draught beer quality in England, Scotland and Wales. In Northern Ireland however, cellar temperatures are typically colder than 12ºC and accordingly draught beer quality may well be better.

Numerous factors will have contributed either positively or negatively to sample quality. Samples from recently cleaned lines, in accounts with good hygienic practices and turnover would be expected to be of better quality than samples from accounts with infrequent line cleaning, a lack of hygienic practices and slow turnover. Accordingly, to mitigate for an 'off day' the majority of accounts were sampled at least twice.

In the work reported here, sampling was during the day between Monday and Friday, with throughputs likely to be lower than in the evening or weekends. However, the beers (SL3, SL6, KA1, KA5) that accounted for the majority of samples (>90%) were all national brands and would be anticipated to have a satisfactory turnover throughout the day. In turn, this was supported by the accounts being centrally located with good passing footfall.

4.4 Conclusions

Draught beer quality is an important factor for consumers, particularly as the price differential of the brand increases between the on- and off-trade. Measurement of quality post dispense, using a validated forcing test *(8),* confirms the widely

held view that draught beer quality is variable. This survey of 57 accounts in the UK Midlands is suggested to be relevant to similar dispense configurations in the UK and elsewhere.

The quality of draught standard lager (abv ≤ 4.2%) ex-trade was found to be superior to keg ale (abv ≤ 4.2%). This is likely to reflect a number of factors including rate of sale and dispense temperature. Overlaid on this, susceptibility to spoilage is influenced by beer composition.

The on-trade account is the major variable that determines good, indifferent or poor beer quality. Implementation of hygienic practices together with well-trained bar staff contribute to the delivery of excellent quality draught beer. Conversely, poor practice and untrained staff will result in compromised beer quality. It is noteworthy, that draught beer quality was found to be inversely related to the number of taps on the bar and that the highest price point is associated with the poorest quality. Further, accreditation of an account to an industry quality scheme for cask beer has no impact on the quality of keg beer.

This work has shown that draught beer quality at the point of dispense is highly variable. Of 237 samples, 34.8% of samples were 'excellent', 42.2% 'acceptable' but 20.3% were 'poor' and 2.7% were 'unacceptable'. As keg beer ex-brewery and pre-dispense should be of 'excellent' quality, it can be argued that 65% of the

keg beers sampled in this work have suffered some microbiological damage as a consequence of dispense. This is disappointing and hopefully will trigger wider studies into draught beer quality. A longer-term aim of this work is that brand owners, retailers and other stakeholders will 'own' the improvement, communication and assurance of draught beer quality.

Future reports will address the impact of hygienic best practice on draught beer quality, importance of throughput, beer composition and variability of spoilage and the impact of brands and accounts on product microflora.

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5 DEFINING BEST PRACTICE AND MEASURING ITS IMPACT

5.1 Introduction

Under the Food Safety (General Food Hygiene) Regulations (1995) beer is defined as a food and hence cellars are subject to the same hygiene standards as commercial kitchens (BBPA briefing, 2018). Much of the anecdotal evidence coupled with the evidence presented in Chapter 4, suggests that standards in cellar management and line hygiene across the U.K. are variable at best.

Unlike the U.K., Germany's Institute for National Standards known at the DIN (Deutsches Institut für Normung) has produced a standard covering all activities related to the dispense of draught beverages including water, soft drinks, wine and beer. The standard (DIN 6650) is made of many component parts covering dispense system design and materials used, through to cleaning and maintenance.

DIN 6650-6 is titled 'Requirement for cleaning and disinfection' and covers all aspects of how to clean and maintain suitable hygiene levels. It refers to levels of contamination in cfu/ml based on traditional plate counts however, does not stipulate the media, sample volumes or incubation ranges to be used.

In the USA, the Brewers Association of America (B.A.) produce annually a draught beer quality manual, setting out a coherent set

of standards for the industry to utilise and adhere to. Specific activities include quarterly acid washing of dispense systems to reduce scale build up, monthly disassembly of fonts and faucets (taps) for full cleaning and inspection and how to conduct a full and proper line clean (Brewers Association, 2019).

Neither of the above initiatives have been replicated in the U.K, and accordingly there is no one industry wide standard. Large brewers, Pub Companies and trade bodies (BII, BBPA and Cask Marque), provide their own less exhaustive guidance.

It is generally accepted that line cleaning of cask and keg beer lines should be performed a minimum of every seven days, cellars should be clean, tidy and temperature controlled at 11-13^{o}C. In the front of house, glasswashers should be subject to routine maintenance and used only for washing of glassware (not coffee cups!) Further, ice machines should be cleaned and maintained, and the removable nozzles/sparklers should be cleaned at the end of service.

Within the U.K., there is no guidance however, on how to measure beer quality, how to validate an effective line clean, or how to sanitise dispense taps, couplers and other components of dispense equipment.

In partnership with a small pub group, beer quality ex dispense was monitored before and after the introduction of best practice

defined as (i) weekly line cleaning with a reputable line cleaner with indicator (Chemisphere Pipeline), (ii) spraying couplers and keg spear with a commercial antimicrobial spray and (iii) daily soaking of removable nozzles in a sanitising solution (Chemisphere). Using the method developed in Chapter 3, any improvement in hygiene/beer quality of the pubs in the trial post best practice could be demonstrated.

5.2 Results and Discussion

Initially beer quality was assessed; at the point of dispense, prior to line cleaning and post line cleaning for three consecutive weeks. The results provided each outlet with a baseline quality level which, was re-assessed after the introduction of best practice, to establish whether there was an uplift in quality. Further assessment(s) performed included direct keg sampling, ATP swabbing of dispense components and comparison of 1st runnings ex tap vs subsequent runnings.

5.2.1 Assessing the 'current' performance of three on-trade accounts

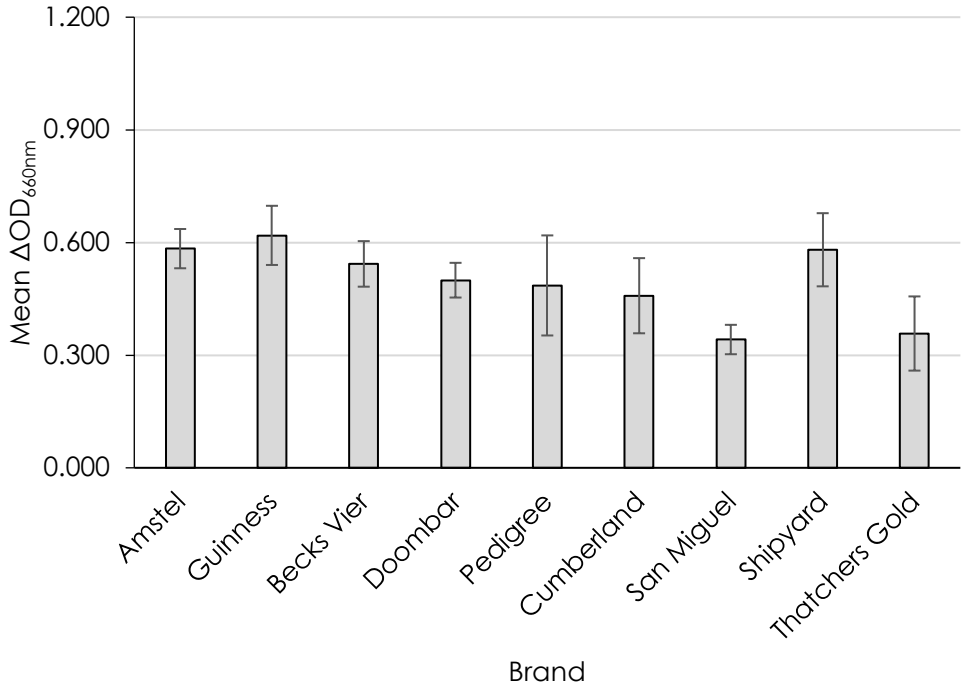
In order to demonstrate any impact of introducing best practice, the base-level quality performance of each outlet was needed. Firstly, each tap on the bar was sampled on three separate occasions and assessed using the forcing test developed in Chapter 3. Figure 5.1 shows the beer quality level for each of the

three accounts: a) The Boardwalk, b) Nags Head and c) The Star respectively.

The Boardwalk had a mean quality index of 74.1% (Table 5.1) across the three visits with each tap regardless of beer style in the 'acceptable' (0.301 ≤ 0.600) or 'poor' (0.600 ≤ 0.900) quality band(s).

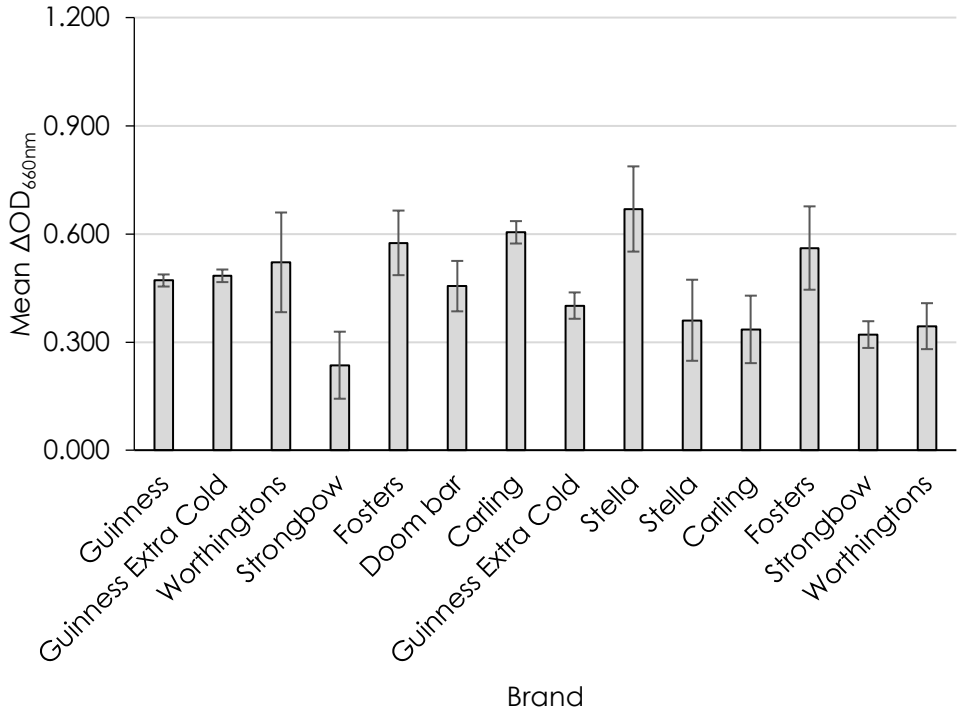
The Nags Head had the most variable quality of the three accounts, with a quality index of 82.1% for the first visit but a significantly lower 67.9% on the third visit. Strongbow was the best performing brand in the 'excellent' category (≤0.300) whilst all other taps were in the acceptable or poor bands. Finally, The Star had the best mean quality index score of 75.8%, with cider and Draught Bass cask beer in the excellent band. The worst performing tap on the bar was fruit cider. Possible reasons for the products poor performance compared to the standard apple cider are likely to be the higher content of sugars and the lower abv (4.7% vs 4.0%). The sugars would provide an obvious nutrient source for spoilage microorganisms, which are typically yeast due to the low pH ≈ 3,0.

3345 a) The Boardwalk



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3347 b) Nags Head



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c) The Star

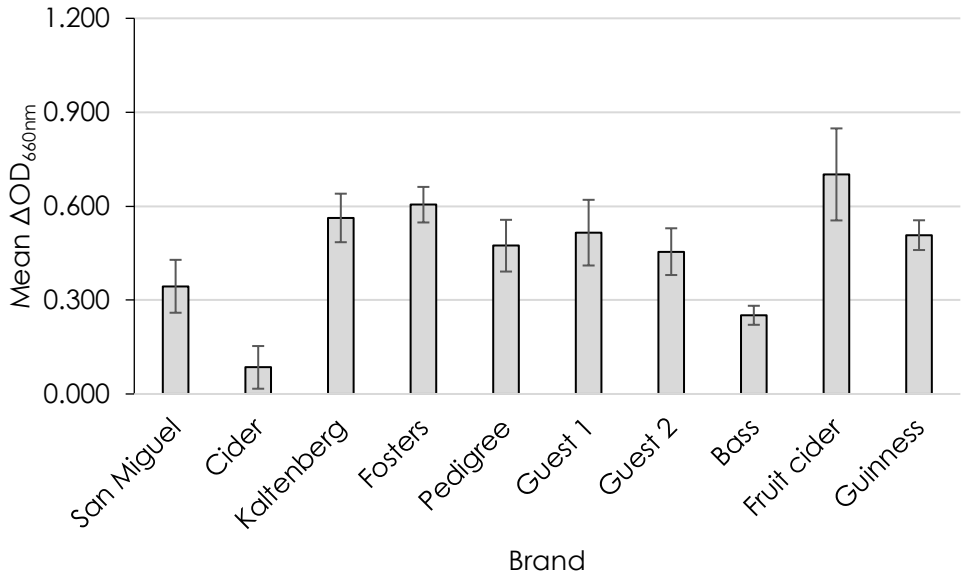


Figure 5.1: Mean beer quality scores ex dispense using the forcing method, for each tap on the bar at; (a) The Boardwalk, (b) Nags Head and (c) The Star, prior to implementing best practice methods. Results shown are from three samplings with standard error bars.

Table 5.1 Quality index scores at the three public houses prior to implementation of best practice.

Public House	Week 1 (%)	Week 2 (%)	Week 3 (%)	Mean
The Boardwalk	77.8	72.2	72.2	74.1
Nags Head	82.1	76.8	67.9	75.6
The Star	72.5	75.0	80.0	75.8

5.2.1.1 Validation of line cleaning

Line cleaning was regularly performed weekly in all three accounts. The general recommendation is to clean lines every 7

days although there is no available explanation why this is the guideline.

For the purpose of this study, the accounts agreed to clean weekly with their in-house beer line cleaner and their current line cleaning protocol. Samples were taken prior to line cleaning, to provide the 'worst-case scenario' for beer quality for each tap. All taps were then sampled again post line cleaning, with the results used to measure the improvement in quality due to cleaning.

At the Boardwalk line cleaning was seen to improve overall quality as the quality index was higher post cleaning for each of the three cleans (Table 5.2). With a mean pre-clean quality of 74.1% increasing to 81.5% after cleaning. Although cleaning was seen to be effective with 90% of taps having better quality post cleaning, the improvement in quality was not deemed statistically significant generating a P-Value of 0.07.

Table 5.2 Quality index (%) pre and post line cleaning at The Boardwalk.

Boardwalk	Clean 1		Clean 2		Clean 3		Mean	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Quality Index (%)	77.8	86.1	72.2	75.0	72.2	83.3	74.1	81.5

Of all taps on the bar only two lines were cleaned such that the product quality was at the excellent level, Thatchers Gold (cider) and San Miguel (premium lager). All other taps had mean values in the acceptable or poor (Amstel lager) bands post cleaning, indicating that cleaning was not 100% effective and could be improved.

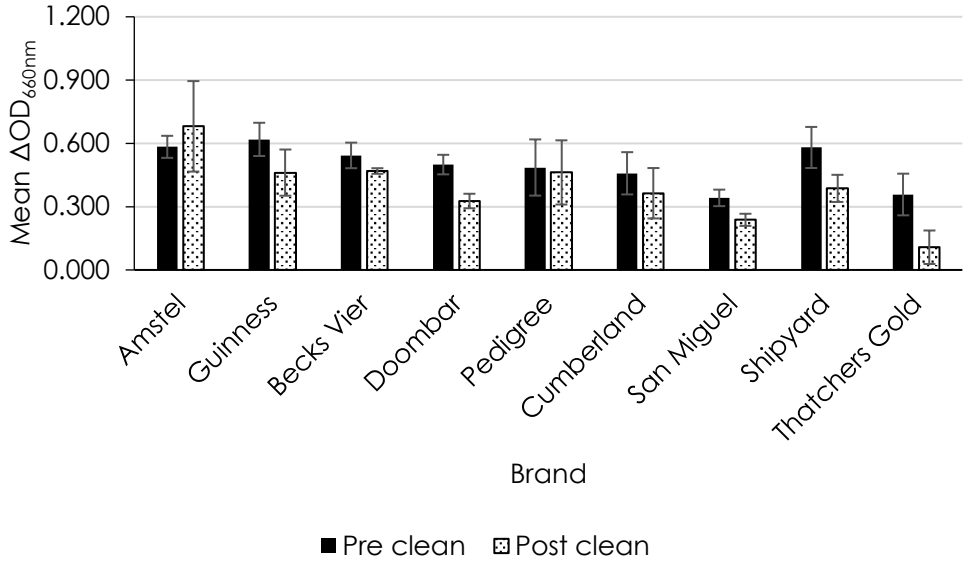


Figure 5.2: Line cleaning at The Boardwalk. Mean absorbance values for each tap, pre- and post-line cleaning for three cleans (triplicate samples with standard error shown).

The Nags Head was the worst performing outlet, with the mean quality index post-clean being lower than that achieved pre-cleaning (Table 5.3). The observation that quality deteriorated after cleaning could be due to poor cleaning practices be it methods and materials or both. The results at the Nags Head are representative of a heavily soiled dispense system, reflecting

ineffective cleaning over an extended period of time leading to a significant and well protected biofilm in the dispense line. It is noteworthy that the Nags Head had the longest dispense lines of the three pubs with the cellar being many metres from the bar. Furthermore, the Nags Head used the largest containers of the pub group (22 gal kegs). Results in Section 5.2.4.4 would suggest these large containers would have a negative impact on beer quality as turnover would be slower and would become reservoirs of microbial contamination. These kegs were also connected with line splitting devices, resulting in multiple lines and taps being fed from the same container. Accordingly, on trading days of low turnover some lines may have been static for long periods of time facilitating the spoilage of the beer within those lines.

Table 5.3 Nags Head quality index (%) pre and post line cleaning.

Nags Head	Clean 1		Clean 2		Clean 3		Mean	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Quality Index (%)	76.8	71.4	67.9	69.6	65.6	66.8	70.1	69.3

The data by tap (Figure 5.3) again showed cider, (Strongbow), to be the best performing brand on the bar. As cider was the best performing brand on the bar in all three accounts, it is clearly more robust to spoilage than beer supporting data presented in Chapter 4. All taps were in the acceptable or poor categories for both pre- and post-cleaning quality whilst one (Carling) had unacceptable

beer quality post cleaning. A P-value of 0.73, for pre vs post cleaning allied with mean ΔOD_{660nm} values of 0.507 and 0.526 for pre and post cleaning respectively highlighted the magnitude of issue(s) at this outlet.

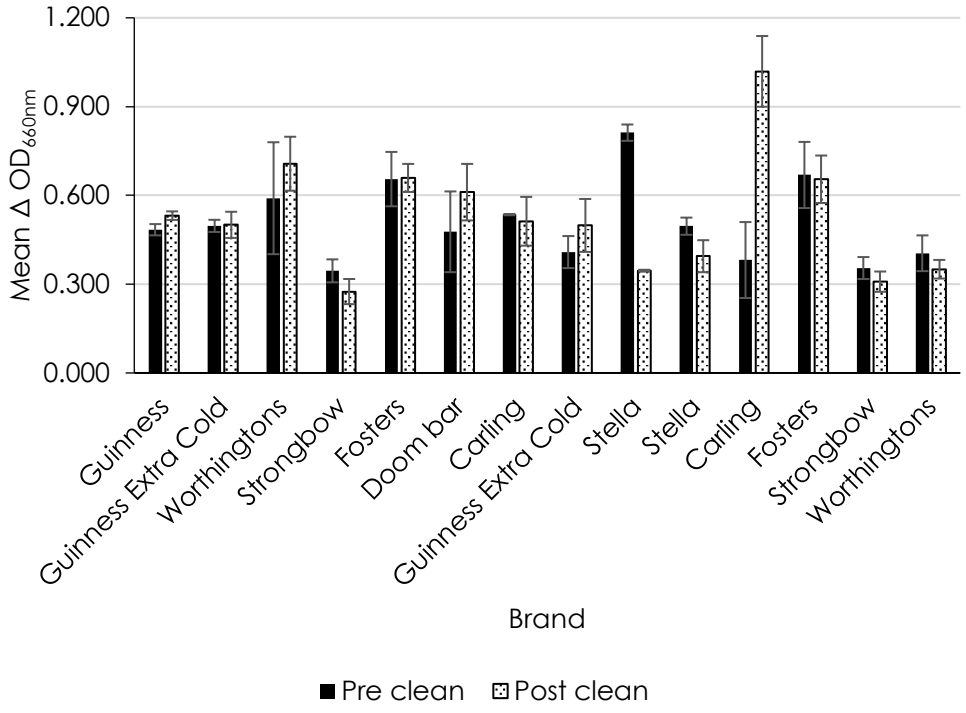


Figure 5.3: Line cleaning performance at the Nags Head. Mean absorbance values per tap for, pre- and post-line cleaning for three cleans (triplicate samples with standard error shown).

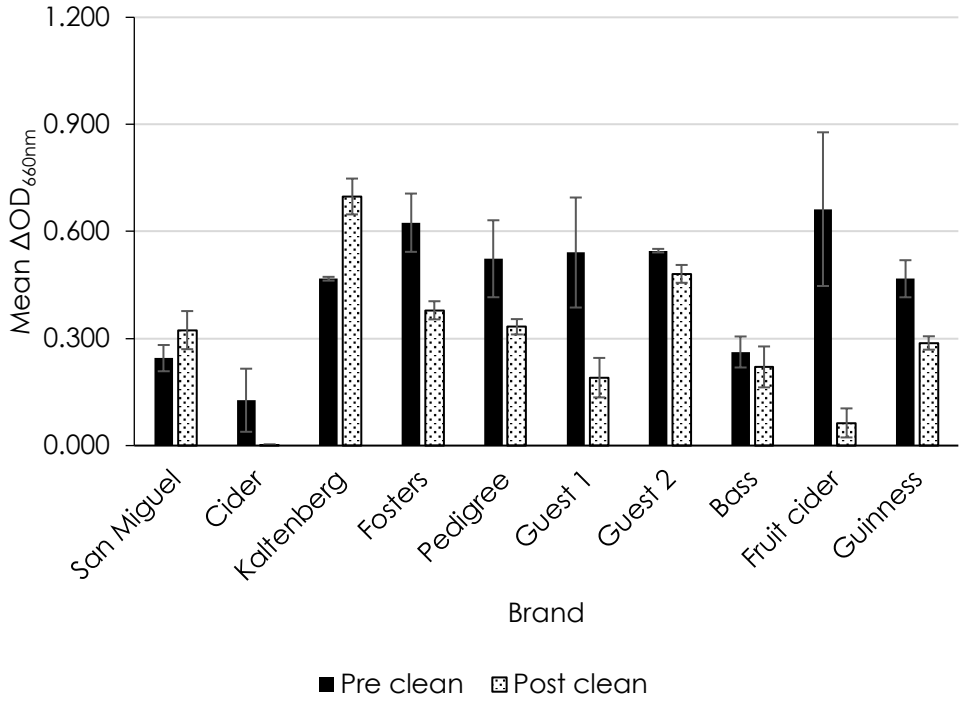
The best quality index (%) was achieved at The Star (Table 5.4). A pre -clean quality of 76.3% and post cleaning quality of 84.6% were the highest of the three accounts. Again, despite achieving the highest quality score in the group, cleaning was not 100% effective as not all beers post-cleaning were in the excellent (<0.300) category.

3429 **Table 5.4** Quality Index (%)pre- and post-line cleaning at The Star.

The Star	Clean 1		Clean 2		Clean 3		Mean	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Quality Index (%)	75.0	85.0	80.0	85.0	74.0	84.0	76.3	84.6

3430

3431 Results by tap (Figure 5.4) shows that on average 30% of taps were
3432 of excellent quality pre -cleaning and 50% were excellent post
3433 cleaning. As found in the other accounts, cider quality was
3434 excellent and exhibited the highest quality of all brands on the bar.



3435

3436 **Figure 5.4**: The Star, line cleaning performance. Mean absorbance
3437 values per tap for, pre- and post-line cleaning for three cleans
3438 (triplicate samples with standard error shown).

Statistical analysis of the absorbance values achieved pre and post clean using a Student's t-test showed significant difference was achieved by cleaning with a P value of 0.03. The Star was the only account of the three to show significant improvement in beer quality due to cleaning prior to best practice introduction.

5.2.1.2 Deep cleaning of three on-trade accounts

Prior to the introduction of best practice, the dispense system in each of the three accounts was intensively cleaned with a Pipeline Bottoming Out kit, which was supplied and performed by Chemisphere U.K. This is a more aggressive line cleaning solution which is used to 'refresh' lines that have not been cleaned for some time or are in a very poor hygienic state. This approach was used to ensure the lines in all three accounts were of comparable cleanliness prior to the trial commencing.

Table 5.5 shows the quality index score(s) achieved after 'deep cleaning', with The Star again performing the best. The Boardwalk results were poorer than expected and - after further investigation of the dispense set-up - it was found that an unused upstairs function bar was still connected to the worst performing three taps on the main bar. These three split lines were the cause of the poor and unacceptable quality results, suggesting that the line feeding the upstairs bar was having a negative impact on the quality of beer in the lines serving the main bar. It was recommended that

the upstairs bar be disconnected. As the quality index for the Boardwalk was 77.8% but with these lines removed rose to 95.8%, suggesting that the intensive clean was effective on all other lines.

There was a notable increase in beer quality at the Nags Head, achieving 85.7% clean and a mean ΔOD_{660nm} in the excellent band at 0.263. An improvement of almost 17% on the beer quality achieved post cleaning previously.

Table 5.5 Number of taps per quality band (A to D) after deep cleaning.

Public House	A	B	C	D	Quality Index (%)	Mean ΔOD 660nm
The Boardwalk	5	1	2	1	77.8*	0.429*
Nags Head	9	5	0	0	85.7	0.263
The Star	7	3	0	0	92.5	0.231

* = results including taps connected to unused function bar lines.

5.2.2 Comparison of quality pre- and post-implementation of best practice

To assess the impact of introducing 'best practice' to the on-trade accounts, three further cleans beginning from one month after the 'deep' clean were measured for beer quality pre- and post-cleaning. The quality index scores were then compared to the scores achieved prior to best practice introduction.

The Nags Head was not part of this experiment as the account had been sold and was no longer available for testing.

Beer quality significantly improved at The Boardwalk (Figure 5.5) after the shift to best practice, the mean pre -cleaning quality index rose from 74.1% to 88.4% and post clean quality increased from 81.5% to 93.2%. As can be seen in Figure 5.5 the worst-case scenario for beer quality (i.e. immediately prior to line cleaning) was now higher than the quality achieved after line cleaning prior to best practice introduction.

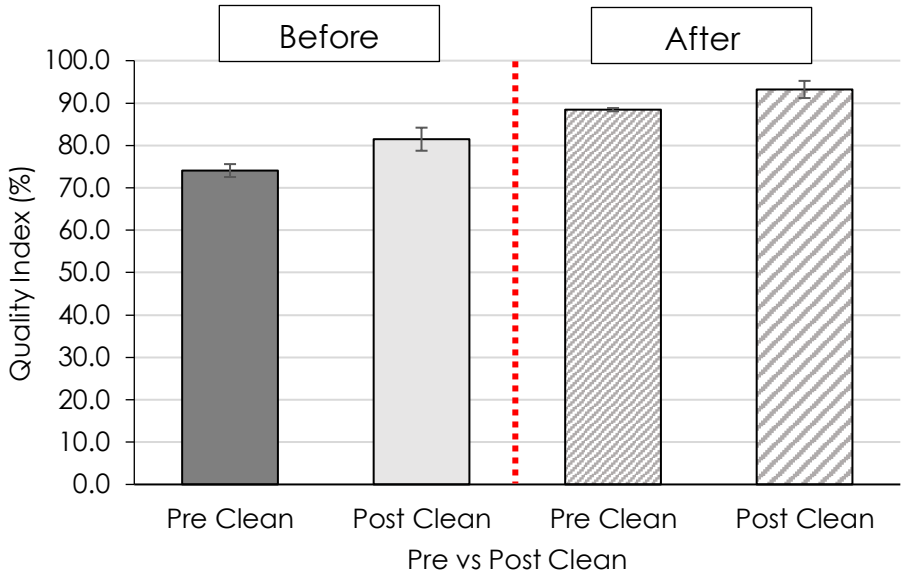


Figure 5.5: The Boardwalk - mean quality index scores pre- vs post-cleaning, before and after the introduction of best practice methods. (Mean of three cleans with standard error shown).

The Star also showed an uplift in beer quality due to best practice however in this case it was not a significant improvement. The comparison of post clean score pre- and post-introduction of best

practice yielded a p-value of 0.18. Despite this it can be seen from Figure 5.6 that beer quality was better both prior to and after cleaning had been performed once best practice methods has been employed by the account.

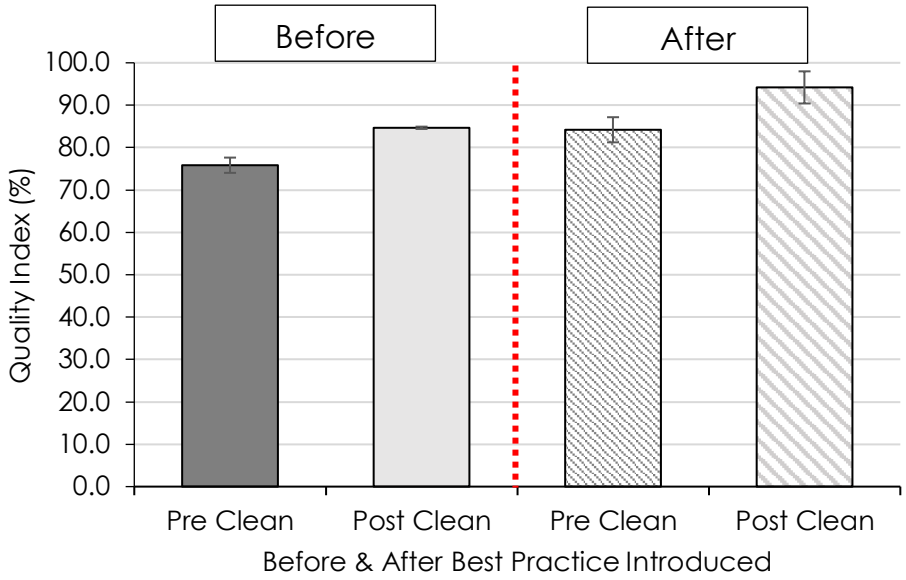


Figure 5.6: The Star – mean quality index scores pre- vs post- cleaning for before and after the introduction of best practice methods. (Mean of three cleans with standard error shown).

5.2.3 Best Practice - dispense tap nozzles and sparklers

Dispense tap(s) are constructed of a font, handle and spout. The spout can be either integral where it is cleaned in situ during line cleaning or more commonly the spout is formed of a removable nozzle (keg) or sparkler (cask).

Nozzles are hygienically vulnerable because they are at the ambient end of the beer dispense system, and suitable for growth of mesophilic microorganisms. Located at the end of the dispense

system they are a potential entry point for environmental microorganisms. Further, nozzles are handled by bar staff (removal, replacement and absent minded 'fiddling'), who may (depending on the premises) also interact with food and introduce cross contamination (Storgårds *et al*., 2000). Cross contamination within brands is also likely since nozzles are mostly universal and many of the taps on the bar will use a similar nozzle type.

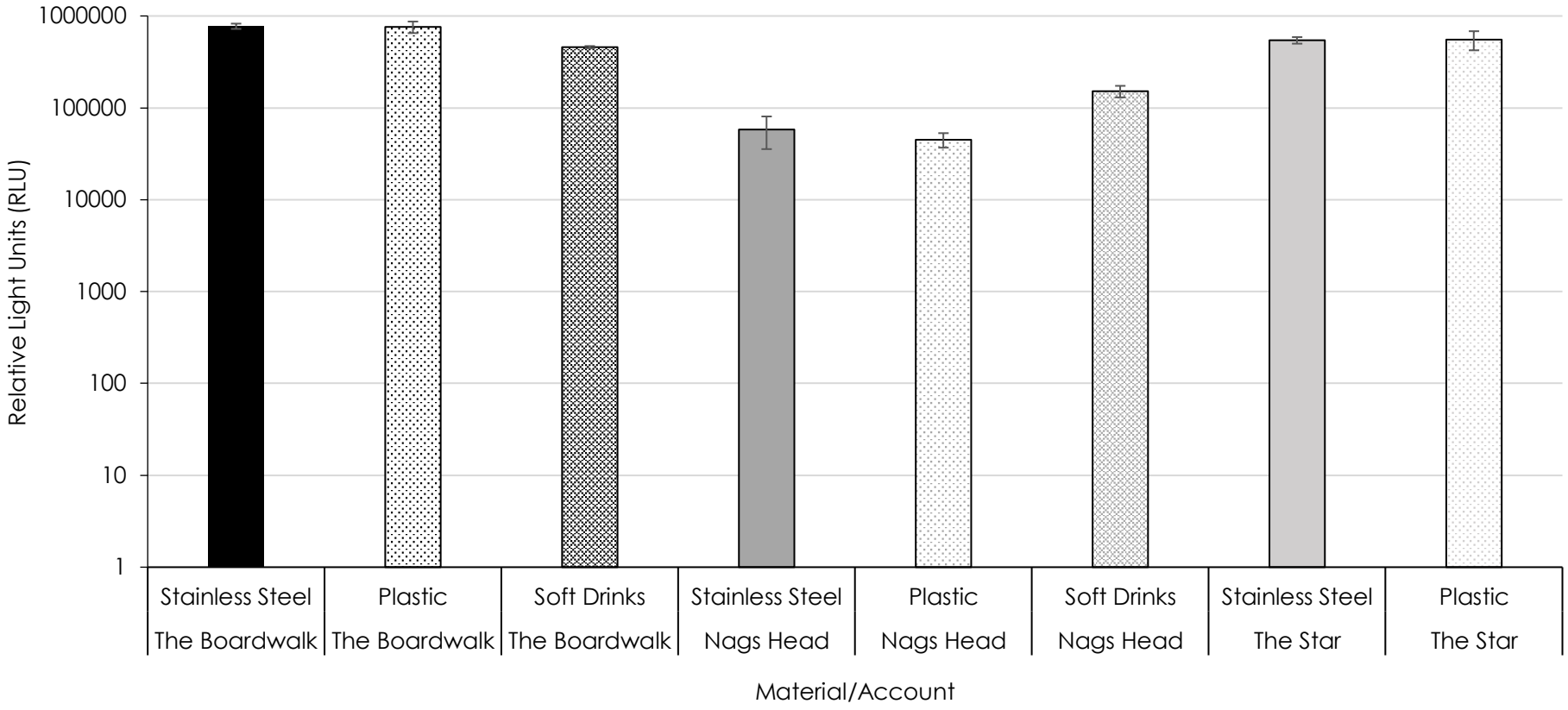
Currently it is recommended by the BBPA that best practice for cleaning of these components involves soaking in line cleaning solution daily at the end of trading (Long, 2003). In practice the vast majority of accounts in the UK soak nozzles using soda water which has been shown to be both ineffective as a cleaner and to exacerbate microbial loading (Quain, 2016). The recommended option of using line cleaning solution is markedly more effective in cleaning nozzles but is not an appropriate option as line cleaning solution constitutes a potential health and safety hazard if kept on or near the bar (Quain, 2016).

All three accounts had nozzles with very poor hygiene. Figure 5.7 shows the bioluminescence (RLUs or Relative light units) of the nozzle surfaces when swabbed for ATP. ATP bioluminescence is a commonly used hygiene monitoring methodology, ATP is present in microorganisms and is used in many biochemical reactions therefore is an excellent indicator of their presence (Webster *et al.*,

1988; Ogden, 1993). The test relies on the reaction between ATP and luciferin which is catalysed by the enzyme luciferase (present in fireflies), this reaction culminates with the production of oxyluciferin which causes the release of a proton which emits a 'yellow-green light' (Rhodes and McElroy, 1958; Siegrist *et al.*, 2015) An acceptable result for clean nozzles would be an RLU of 250 or lower (Storgards and Haikara, 1996). whereas all nozzles regardless of material (plastic or stainless steel) or account had markedly higher values than this. Indeed, the Boardwalk was the worst performing account with RLU levels just below 1,000,000.

Following the recommendations published by Quain (2016), the three accounts were provided with sanitising tablets from Chemisphere UK and instructed to use them overnight after each day of trading by placing all nozzles in a pint (568 mL) glass of cold water together with a single tablet.

Table 5.6 shows the RLUs achieved after swabbing the daily sanitised tablets from The Boardwalk. A significant improvement in the hygiene of the nozzles was achieved, comparable to that reported by Quain (2016). Here, stainless steel nozzles showed a 10^4 reduction in RLU values ($p<0.001$) and plastic nozzles a 10^3 reduction with a p value <0.05.



3556

3557 **Figure 5.7**: ATP Bioluminescence as relative light unit(s) for swabbing of nozzles, split by material and account. Duplicate

3558 samples with standard error bars shown against a logarithmic y-axis.

The sanitising tablets contain sodium dichloroisocyanurate (sodium troclosene) which is a water soluble white solid and when dissolved in water results in a slow release of chlorine making it an effective sanitiser used in water treatment and swimming pools.

Chlorine based cleaners are known to have the potential to cause beer taints due to the reaction of chlorine and phenols present in the beer, forming compounds known as chlorophenols which have a relatively low flavour threshold (300 ng/L)., To prevent this nozzles were rinsed with fresh cold water prior to use for trading.

Table 5.6 RLU level comparison between two treatments; Soda water soak and Sanitising tablet in a pint (568 mL) of cold water at The Boardwalk.

Public House	Treatment	Material	Mean RLU	95% C.I.	P. value
The Boardwalk	Soda Water	Stainless Steel	774,295	± 100,149	P<0.001
The Boardwalk	Sanitising tablet	Stainless Steel	139	± 114	
The Boardwalk	Soda Water	Plastic	760,093	± 214,727	P<0.05
The Boardwalk	Sanitising tablet	Plastic	691	± 663	

5.2.4 Best practice - impact of dispense throughput

The throughput rate of beer will vary for a plethora of reasons such as brand popularity, pricing, day of the week, footfall, events and the number of taps on the bar. It is recommended that a single keg be emptied within 5 days and a cask beer be on sale for no longer than 3 days once tapped and vented (Nelson *et al.*, 2018).

Best practice suggests that the full line volume from cellar to bar be emptied prior to any trading session. The beer that has been stationary in the line overnight, will have been in contact with the microorganisms attached to the beer line for an extended period. Hence beer quality is most likely to have been compromised by the formation of spoilage products such as acetic acid, lactic acid, diacetyl, phenolics etc.

5.2.4.1 Dissolved oxygen

To assure flavour stability, dissolved oxygen across the process and in packaged product is controlled to low levels (< 100 µg/L). However, despite this attention, dissolved oxygen in draught beer has not been reported. That said, innovations in beer dispense lines have focussed on reducing oxygen pick up and loss of carbon dioxide. Accordingly, dependent on the line material, the level of dissolved oxygen in the beer in the line will be markedly higher than that in the 'anaerobic' keg. It would be anticipated that elevated levels of dissolved oxygen will cause the production of staling

compounds and off flavours such as acetaldehyde (oxidation of ethanol). To test this, using a bespoke dispense rig built by Diageo GB levels of dissolved gases were measured in dispense lines made of two different materials (Table 5.7).

Table 5.7 Level of dissolved gases within a beer line by volume dispensed.

Brand and line type	Hop House 13 Medium density polyethylene			Hop House 13 Barriermaster Flavourlock		
Volume (mL)	200	500	1000	200	500	1000
Dissolved O_2	3.87	7.4	0.64	1.32	0.16	0.11
Dissolved CO_2	1.75	1.86	2.07	2.00	2.06	2.06
Temperature °C	6.3	8.2	3.9	6.4	4.5	3.6

Clear differences were seen between the two different line materials, the medium density polyethylene (MDP) line contained raised dissolved O_2 (DO) levels at all three sample points when compared with the Barriermaster Flavourlock (FL) line. The DO level in the MDP line peaked at 7.4 mg/L after 500 mL was dispensed whilst at the same point the FL line contained just 0.16 mg/L. Conversely the dissolved CO_2 levels were both consistent and higher in the FL line than those observed in the MDP line.

These differences suggest that the Barriermaster Flavourlock line is a better retainer of dissolved CO_2 and its construction prevents the ingress of DO whilst, the traditionally used MDP line is more permeable and less temperature stable. An increased level of DO coupled with warmer beer temperature would cause accelerated oxidation of the product.

5.2.4.2 Dispense 1st Runnings

A 1st runnings investigation was performed in the three on-trade accounts, with consecutive samples (250 mL) taken from each tap before the account opened. The quality of the first aliquot (1st runnings) was compared to the 2nd sample of 250 mL (see Figure 5.8).

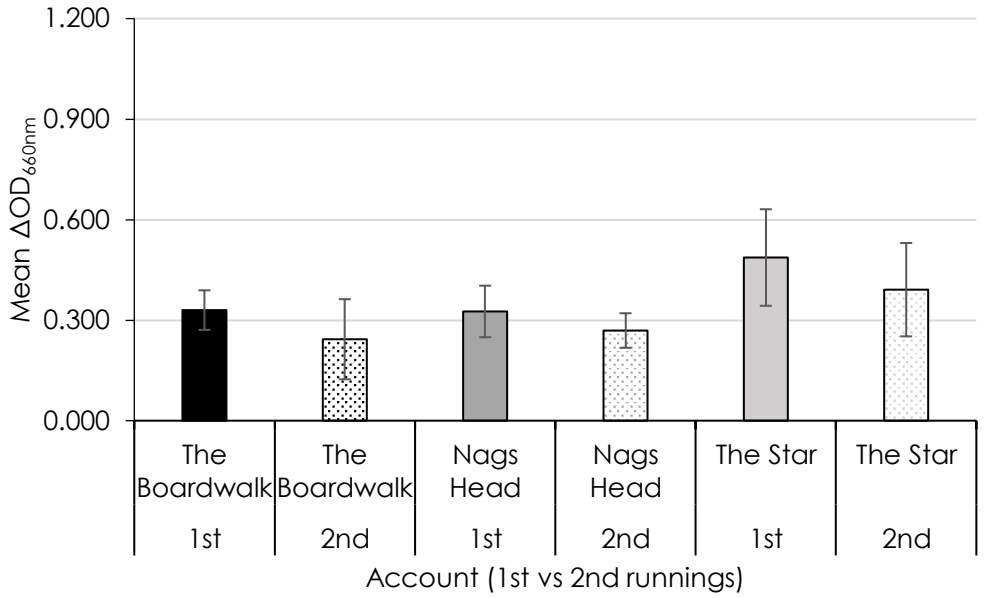


Figure 5.8: Mean quality score of all taps for 1st and 2nd runnings prior to a trading session. Triplicate samples with 95% confidence interval limits shown by error bars.

In each account the 1st runnings were of poorer quality than the second sample, indicative of the increased microbial loading of the beer left in the line overnight, aided by an ambient temperature environment with higher dissolved oxygen levels. Such conditions are ideal for microorganisms such as yeasts and acetic acid bacteria to grow.

However, the quality was not significantly improved in the second aliquot of beer, with p-values of 0.25, 0.29 and 0.39 for each account. This is likely to be due to the varying line length from cellar to bar in the three accounts and accordingly the second sample will also have been sat in the line overnight depending on the length/volume of each line.

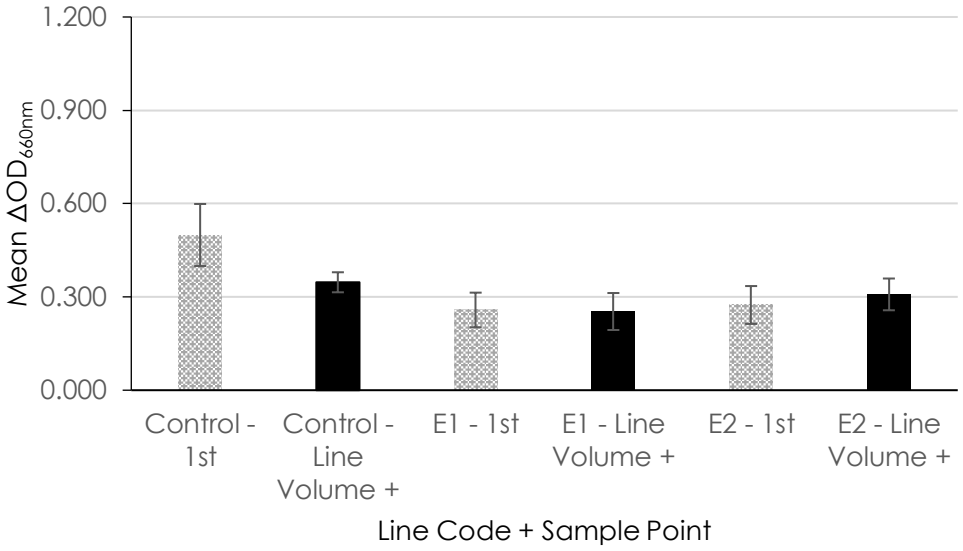
The sampling plan for this initial investigation didn't consider this variance in line volumes and therefore the results are indicative of a pattern but not truly representative of 1st runnings against secondary runnings, as (ideally) the entire line volume should be emptied prior to trading.

A further investigation was performed using the dispense rig designed and installed by Molson Coors. By using the dispense rig in which all lines had a similar length, therefore holding the same line volume, the quality of 1st runnings could be more effectively tested. Again the 1st runnings were the first 250 mL from the tap after the system had been static overnight, followed by the total

volume of the line being emptied after which a fresh sample (250 ml) of beer was taken.

Samples were taken on day four and day seven for three consecutive weeks. Used kegs were replaced with fresh kegs after samples were taken on day seven of each week. Figure 5.9 shows the results for 1st runnings vs post line purge of four-day old (A) and seven-day old (B) keg(s). A significant difference was seen between the 1st runnings and the beer sampled post line purge in the control line only at both 4 and 7 days. This line on the dispense rig is representative of dispense systems across the U.K., with the temperature at 6-8°C and the line material of medium density polyethylene (MDP), with a standard foam on beer (FOB) detector fitted in the cellar.

A



B)

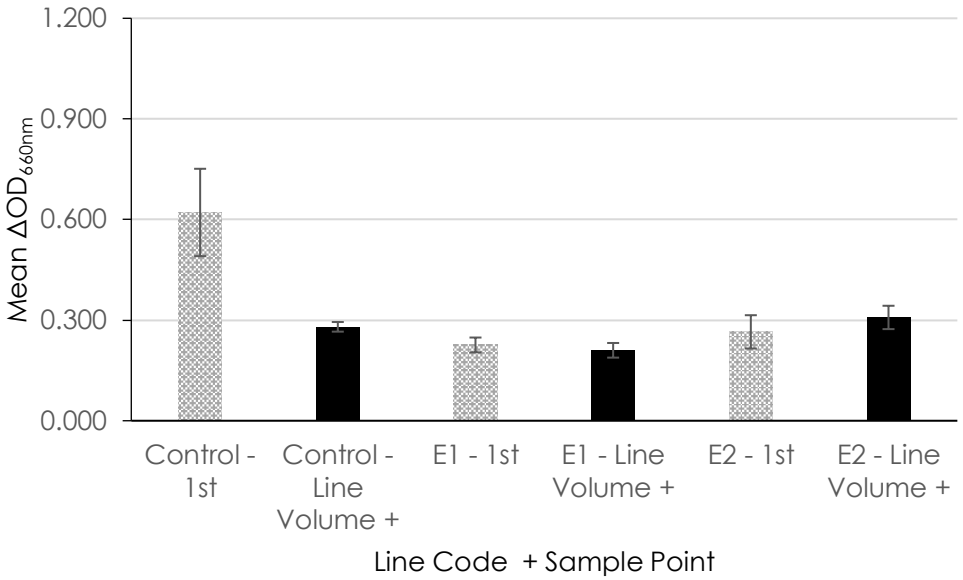


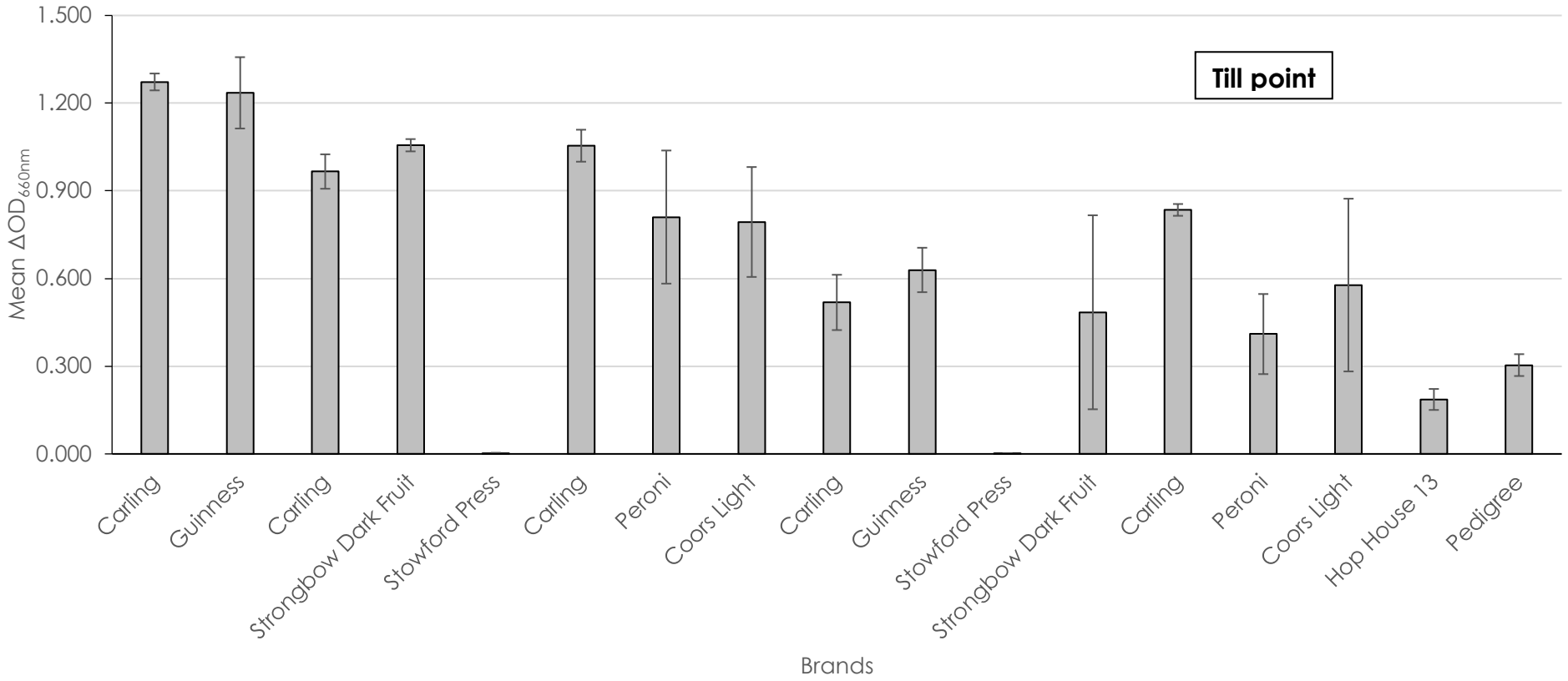
Figure 5.9: Absorbance increase measured at OD_{660nm} for 1st runnings samples vs. samples taken after emptying the full line volume from three different lines at A) four days and B) seven days, on a bespoke laboratory built dispense system. Triplicate samples taken with 95% Confidence interval shown by error bars.

Of note is the decrease in quality of 1st runnings at day 7 compared to day 4, suggesting the increased spoilage potential over a 7 day period. This suggests cleaning at 7 days is appropriate for best practice. However, keg age may also have an effect and this is explored in Section 5.2.4.4.

5.2.4.3 Best Practice – over-fonting and the 'hotspot'

Further complexity is added to dispense systems in public houses with the addition of too many taps and splitting of beer lines after the FOB, so as to feed multiple taps from a single keg. A large number of taps may be installed based on venue size and to cater for expected busy periods arising across the weekend. However, many of these taps are then often redundant during the quieter weekdays and - as seen from the 1st runnings investigation - if beer is sat in a line for a protracted period during the week, the quality of the beer will be increasingly poor.

Figure 5.10 shows beer quality across the bar of a high street outlet, with the location of the till shown. The quality of dispensed beer deteriorates along the bar as the taps are further from the till sited opposite the entrance to the outlet which is where customers are served in quiet periods. Samples were taken on a Thursday prior to cleaning with 29.4% of taps being of unacceptable quality (>0.900).



3696

3697 **Figure 5.10**: Beer quality across the bar at a high street account, The Locomotive. Triplicate samples showing standard error.

Further evidence of having too many taps (termed 'over-fonting') is seen at the Nags Head (Figure 5.11) Duplicate taps are positioned side by side on this bar and fed from the same keg, again in all cases one tap is of better quality than the other however, there is only significant difference seen between the two Carling taps. Extra complexity in the dispense system coupled with increased tap numbers will have a detrimental effect on beer quality by re-seeding of the line from that tap that is less used, coupled with a reduction in the throughput(s) through each tap causing extended periods where beer is static which allows beer quality to deteriorate (see Section 5.2.4.2)

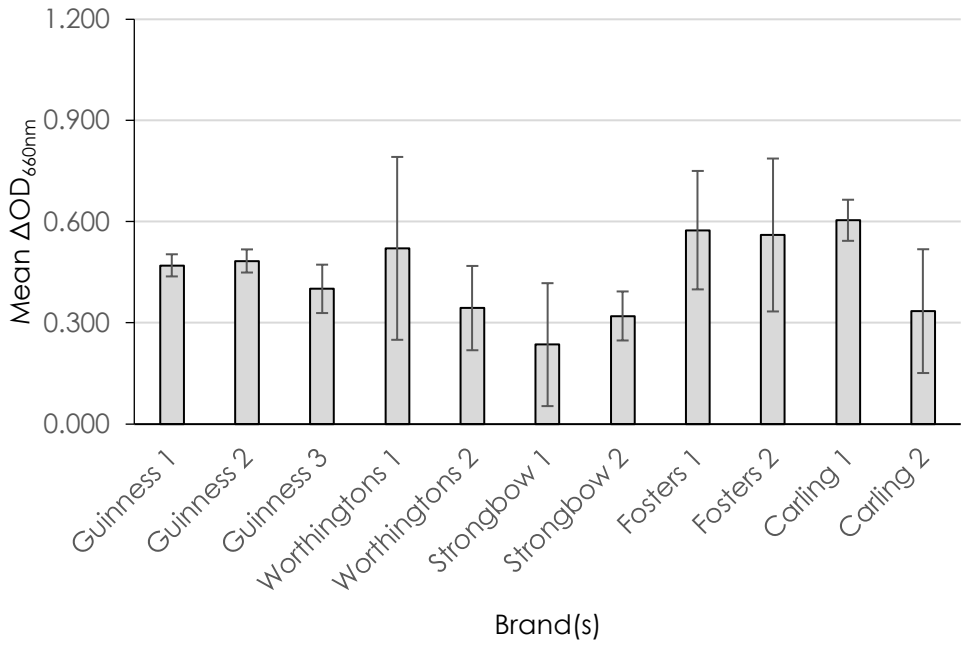


Figure 5.11: Mean dispense quality for each tap at the Nags Head public house, triplicate samples with 95% confidence interval error bars shown.

Another outcome of over-fonting was observed again, at the Locomotive. A number of the samples taken after performing a line clean were in the poor or unacceptable quality bands. Figure 5.12 shows the results of two cleans at the locomotive, it shows that the Carling taps were not being effectively cleaned and were in fact of poorer quality after cleaning. Again, the Carling tap furthest from the till recorded the poorest quality score.

Figure 5.13 shows all other taps on the bar were being cleaned effectively with a quality index score of 95.8 and 97.9% being achieved for each of the two cleans respectively (Table 5.8).

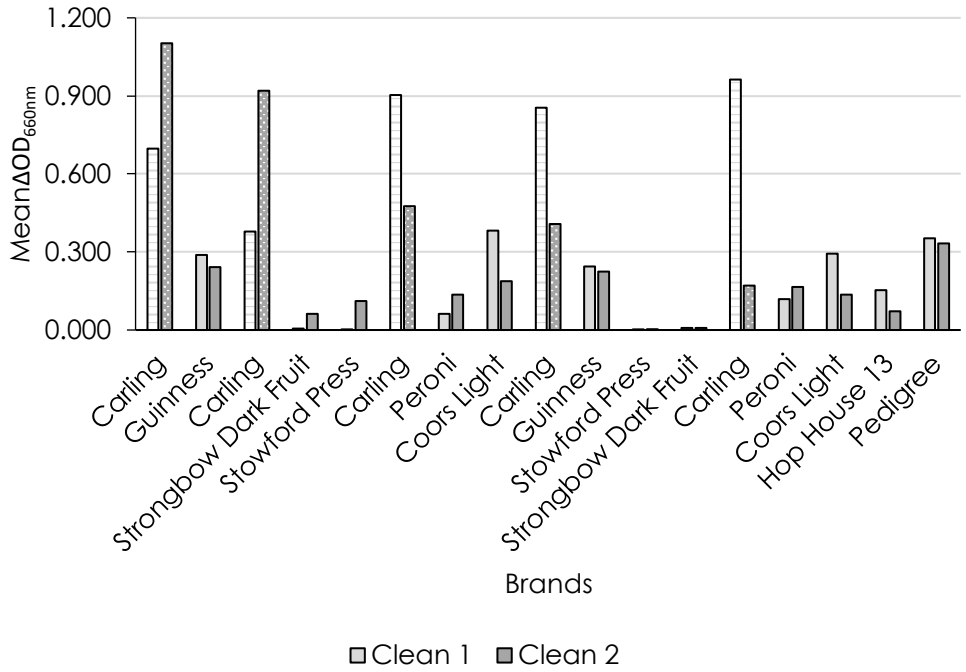
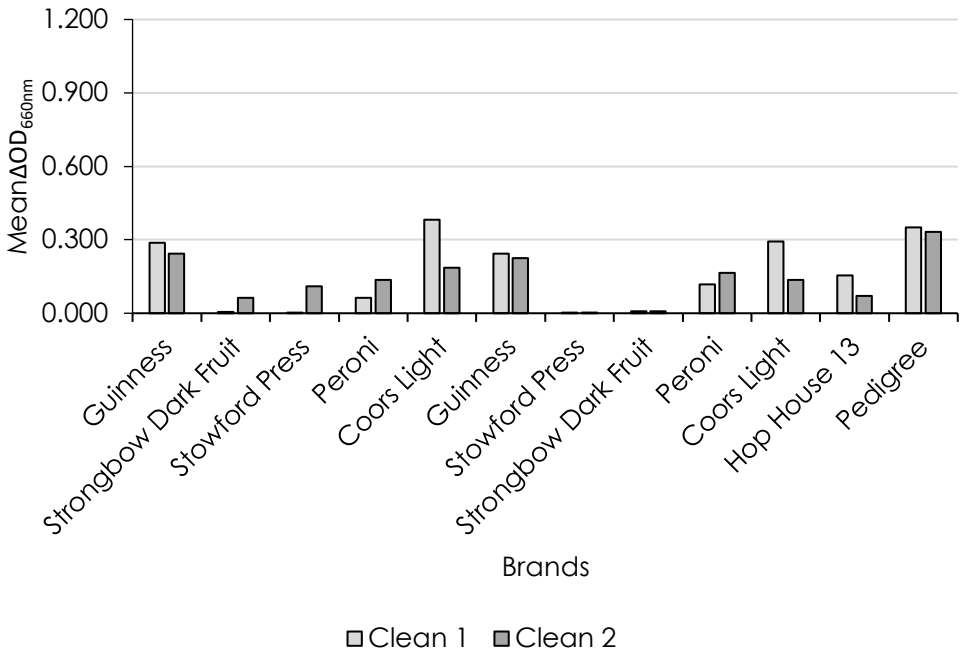


Figure 5.12: Beer quality achieved for each tap on the bar, post line cleaning. Triplicate sampling of two cleans.



3727

3728 **Figure 5.13**: Beer quality achieved with Carling taps removed from
3729 the bar.

3730 **Table 5.8** Comparison of post cleaning results achieved with and
3731 without Carling taps being included.

	Clean	No. A	No. B	No. C	No. D	Q.I. (%)
With Carling	1	10	3	3	1	82.4
	2	12	3	0	2	86.8
Without Carling	1	10	2	0	0	95.8
	2	11	1	0	0	97.9

3732

3733 After further discussion with the account and an observation of
3734 their line clean, it was seen that there was no cleaning ring main
3735 present in the cellar. Instead, a single pipe which was fed from

both ends was used for cleaning. This meant that line cleaner and subsequent rinse water exited through the lines located at the centre, which were the Carling lines. Effectively the Carling lines were being re-contaminated with the washing from all other lines on the bar. Compounding this issue was the complexity of the beer lines, five Carling taps were being fed from three Carling kegs. Therefore, there was increased complexity in the Carling dispense lines and likely more 'dead legs', as was noted at 'The Boardwalk' (see 5.2.1.2) lines less regularly used likely leads to increased spoilage of the partner line.

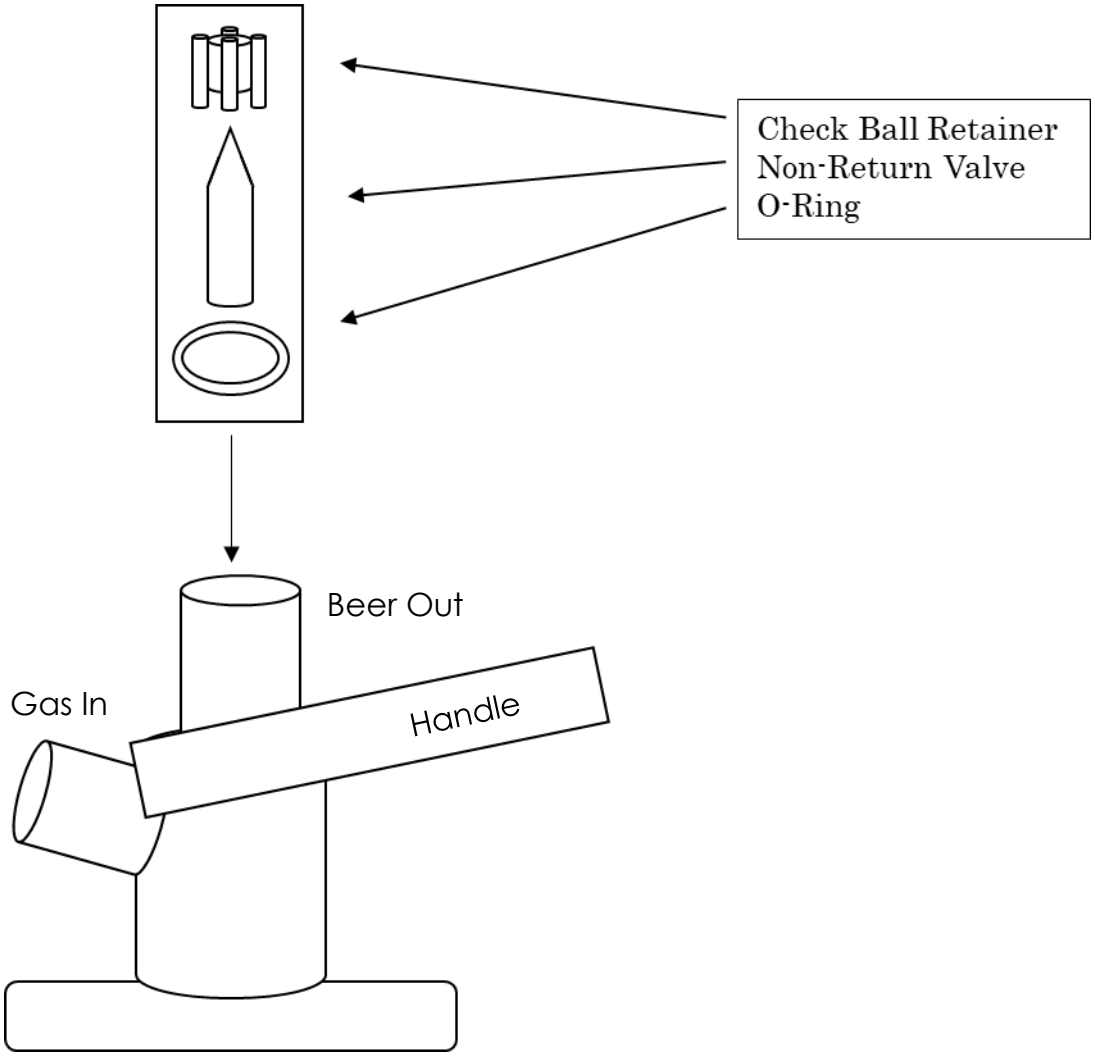
Furthermore, the keg size used for Carling at the Locomotive was 22 gal. this provided a vast reservoir for microorganism growth (see 5.2.4.4). This keg size was selected for expected busy periods over the weekend trading sessions.

5.2.4.4 Contamination of kegs upon connection to a dispense system

After cleaning, kegs are re-connected to the dispense system. Best practice recommends sanitisation of the keg spear and coupler to prevent ingress of microorganisms from the cellar environment. Kegs are filled under pressure to aid in the carbonation of the beer inside the keg and to drive beer dispense. Accordingly, the pressure is higher inside the keg, so the beer is forced out up the spear as dispense gas is pushed in which should act as a barrier to beer moving back down the line into the keg.

Inside a keg connection device, 'keg coupler' there is a non-return float control (Figure 5.14) and as the tap is opened the float control rises allowing beer to move out of the keg and through the coupler into the beer line on route to the tap. Upon closing the tap, the flow of beer at pressure is stopped and the float control settles back down into the closed position.

Figure 5.14: Diagram of a keg coupler non-return valve.



As shown in Section 5.2.1 after line cleaning not all the dispensed beers are in the 'excellent' (<0.300) category, suggesting that the

cleaning methodology could be improved. However, there is little or no understanding of the impact on quality from the re-attachment of the previously connected, broached keg. As it is highly unlikely that all kegs will have emptied at the same time as the line clean is due, any contamination in the kegs will re-infect the line post cleaning.

It is accepted that kegs leave the brewery in a commercially sterile state, and contain <1 cfu/mL (Boulton and Quain, 2001). But there is no published data about the contamination of kegs upon connection to a dispense system.

An initial investigation (Figure 5.15) was performed using the Diageo dispense rig. Kegs were sampled prior to connection to the dispense system and then at 4, 7 and 14 days. The kegs were kept in an ambient environment with no external cooling.

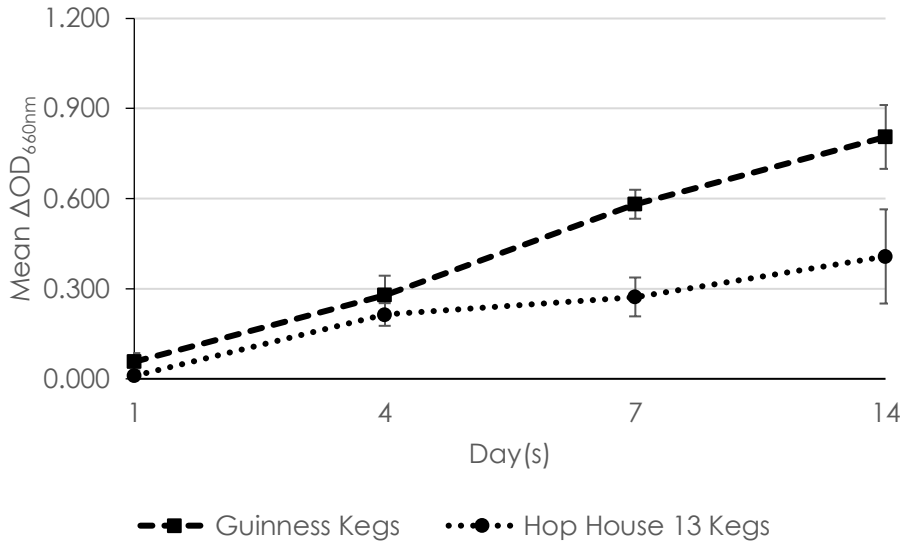


Figure 5.15: Deterioration in quality of beer in keg upon connection to a dispense system. Mean values for growth in keg shown (8 kegs per brand, triplicate samples). Error bars show 95% confidence interval.

All kegs prior to connection were of excellent quality with mean ΔOD_{660nm} values of 0.057 (Guinness) and 0.010 (Hop House 13). After 4 days there is significantly more growth in the kegs for both brands, and after 1 week, Guinness kegs have deteriorated to just below the poor quality level with a mean value of 0.581 whilst Hop House 13 kegs have also deteriorated but remain within the 'excellent' band (mean value of 0.273).

The results seen in this initial investigation suggest that movement of beer is not exclusively one way and that the float control within the keg coupler is not hygienically designed. The turbulence during beer dispense will disturb sessile cells and loosely attached fragments of biofilm within the beer line. Upon closing the tap, the beer flow is suddenly stopped causing a ripple effect back down the beer line and hence a small volume of beer will re-enter the keg prior to the float control closing. Consequently, the more occasions where the tap is opened and closed results in more opportunities for spoilage organisms to enter the keg (Figure 5.16). All couplers regardless of type contain a similar float control system. So, it is a reasonable assumption that all keg couplers allow the re-entry of beer into kegs upon closing of the tap.

Growth of microorganisms will be slower at cooler temperatures, so this investigation with kegs held at ambient temperature represents a worst-case scenario. However, it is accepted that cellar cooling in the U.K. is ineffective at keeping cellar temperatures at the target 11-13°C especially during the summer months.

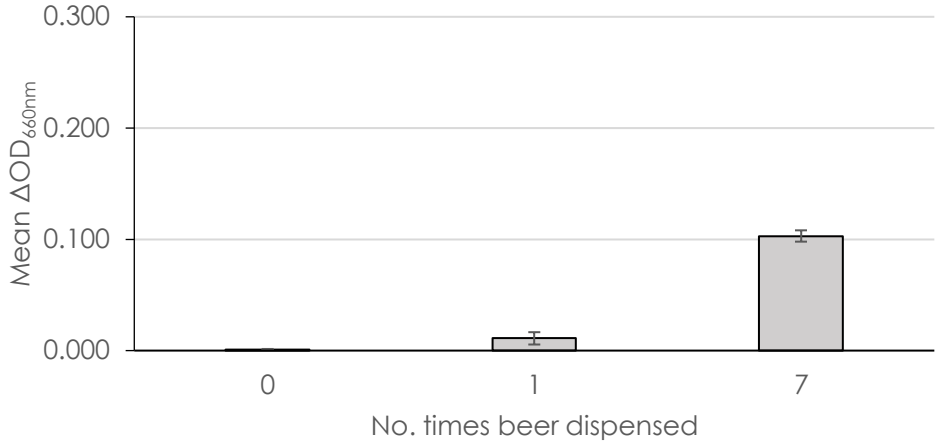


Figure 5.16: The effect of increased number of dispense tap opening and closing cycles on beer quality in keg. Triplicate samples with standard error shown.

To validate the sampling method (see method 2.1.3) kegs were routinely sampled for two weeks without connection to the dispense system and then again for a further week upon connection to the dispense system (Figure 5.17). For this assessment kegs were stored at ambient and also cooled by use of an insulating keg jacket that was connected to a remote cooler. Beer sampled from ambient kegs was at 20.6°C ± 0.3°C whilst beer from jacketed kegs was measured at 14.4°C ± 0.5°C.

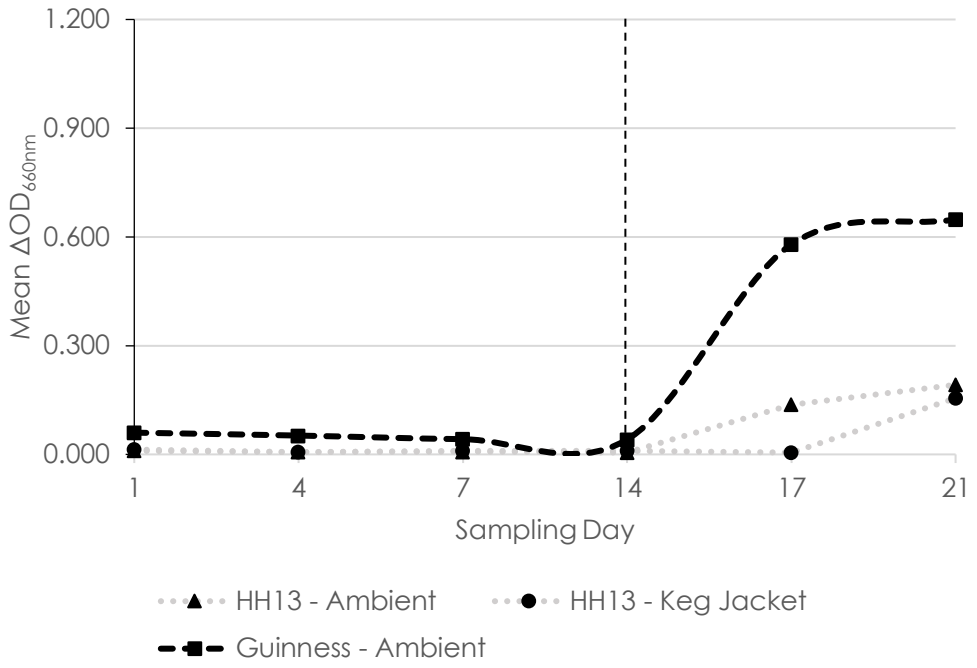


Figure 5.17: Keg quality pre- and post-connection to a dispense system, two weeks period prior to connection and one-week period post connection.

Beer quality is consistent and unaffected by four rounds of sampling, providing confidence that the sampling technique was not contributing contamination to the keg.

Upon connection to the dispense system, the kegs deteriorate in quality with Guinness declining at a significantly higher rate than Hop House 13. The cooling jacket suggested that reduced temperature storage extended the period in which keg quality was unaffected. However, by day 7 quality in the cooled keg had declined to a similar level to that of the ambiently stored keg. A lower storage temperature will reduce the rate of spoilage but the action by which the microorganisms are entering the keg is

unaffected by the introduction of the keg jacket. Beer quality at the cooler temperature, like that of a U.K. cellar deteriorates by day 7 which is in line with the recommendation that a keg is emptied every 5-7 days.

In Ireland and the United States, cellars are much cooler and designed for keg dispense, the cask beer legacy in the U.K. has resulted in cellars which are not refrigerated to the same extent and this will have a negative impact on maintaining beer quality in keg.

5.3 Conclusions

In this Chapter the key concepts of best practice for beer dispense were identified and evaluated. By forming a close working relationship with three on-trade accounts a range of variables could be tested in the real-world.

To successfully measure a demonstrable impact from the introduction of best practice, beer quality was measured across the bar for a period of three cleans, giving a mean quality index (%) for all three accounts. Initially the three accounts were achieving a score of 74-76% which increased to 80-85% after line cleaning.

Introduction of best practice improved beer quality significantly in The Boardwalk and to a lesser degree at The Star (but not significantly). It should be noted that The Star was the best

performing account prior to best practice methods being employed. Unfortunately, due to a change of ownership this work could not be continued to completion at the Nags Head.

Work in the on-trade led to further investigation of key concepts associated with the best practice of beer dispense. Line cleaning was rarely seen to achieve consistently 'excellent' results (<0.300) across all taps on the bar, despite all lines being treated the same. To understand this the hygienic status of kegs was investigated that had been attached to bespoke dispense systems built on site at the University by Diageo GB and latterly Molson Coors brewing company. Kegs are shipped from the brewery in a commercially sterile state (<1 cfu/mL) (Boulton and Quain, 2001) and data reported in this Chapter shows that upon connection to a dispense system there is the potential for microorganisms to move from the beer line into the keg via the keg coupler and spear. It is suggested this is due to the nature of the 'non-return' valve encompassed within the structure of all styles of keg coupler(s) used today. Further support for this is given by the greater contamination found in a keg that has been dispensed on multiple occasions than one which has been dispensed from on a single occasion. This again suggests the increasing ingress of beer back into the keg increased opening and closing of the tap.

After line cleaning the reconnection of contaminated kegs acts a source of microorganisms to re-seed the beer line(s). It is suggested that this is one of the unappreciated causes of declining beer quality seen across the seven-day recommended cleaning cycle. A cellar temperature of 11-13^{o}C will reduce the speed of keg contaminant growth but not sufficiently to extend the cleaning period.

The removal of 1st runnings prior to the start of a trading session was once normal practice but in recent years with accounts facing greater financial pressure the ability to sell - rather than dispose of the beer that has been sat in the beer line is appealing. However, the beer is of poorer quality and could accordingly have a detrimental impact on the outlet reputation if customers are repeatedly served poor quality beer.

The throughput of beer should ideally be sufficient to ensure keg turnover through a five to seven-day period. Dispense line complexity should be reduced where possible, results reported here suggest accounts with a high number of taps - referred to as 'over-fonting' - can suffer from declining quality in taps of the same brand across the bar, the quality of which can also be negatively impacted by the distance from the 'hotspot' on the bar (where customers and bar staff naturally gravitate).

Finally, hygienic handling of dispense system components, notably removable nozzles and sparklers was also studied and the results agree with previous work (Quain, 2016). This reinforces the need for proactive sanitation of nozzles located at the 'end' of the dispense system which at ambient conditions are a major entry point for beer line contaminants.

6 HYGIENIC EVALUATION OF A NOVEL DISPENSE SYSTEM AND CLEANING PRACTICES – MOLSON COORS UK

6.1 Introduction

Following the release of the Heineken SMART Dispense system and Carlsberg's quality dispense system (CQDS) into the on-trade, Molson Coors designed a novel dispense control system referred to as project 'Houston'.

The Heineken SMART dispense was released in 2013, offering a rented dispense system that chills beer on exit from the keg all the

way to the tap so as to ensure a consistent pour at ≤3 °C. This approach was designed to reduce the contamination from microorganisms and maintain good beer quality for longer. Further, at the time, it was suggested that cellars need not be cooled with the kegs stored at ambient temperature. In addition, as part of the offer to licensees, Heineken technicians would visit each outlet and perform line cleaning thereby removing the responsibility from the outlet.

Similarly, to SMART dispense, Carlsberg's CQDS, launched in 2017, was designed to chill from keg through to tap with a recommended clean every 28 days. However, unlike the Heineken system, this is performed by the outlet and not the Carlsberg dispense technicians.

Neither company have published any data regarding the quality of products dispensed through their new dispense systems. The low temperature of ≤ 3°C would of course slow the rate of growth of mesophilic microorganisms (Ingraham and Bailey, 1959; Ratkowsky *et al.*, 1982). However, there is still the potential for ingress of microorganisms into kegs at ambient temperature providing a reservoir of contaminated beer (Section 5.2.4.4). Most likely because of this threat, the Heineken proposition was revised, and kegs must be kept in a conventional cellar at 12°C.

The initial design work for project Houston centred on ensuring correct gas pressures and temperatures were achieved. This would lead to a reduction in fobbing and subsequent beer wastage which was identified as key issue for accounts (Quain, 2006). The control unit regulates and adapts to changes in pressure as the keg empties, reducing the need for adjustment by outlet staff.

The system variables studied in this work were line material, font type (cooled or non-cooled) and the temperature of dispense; standard (6-8^{o}C) vs. extra cold (1-4^{o}C). Further, an extension to the cleaning frequency was validated alongside a comparison study of four different line cleaning brands.

Experimental design:

A single control line was installed which replicated the current standard UK dispense setup, with medium density polyethylene beer line and cooling (6-8^{o}C) from the remote cooler to the tap. Alongside this were four standard temperature lines and four extra cold lines of the new design. The full setup and schematic can be seen in section 2.5.1.2.

Consecutive periods of dispense were sampled and tested for beer quality at the point of dispense, beer quality in keg and biofilm formation. Beer volatiles were also analysed when evaluating first runnings and keg quality. All testing was performed in triplicate for statistical robustness.

6.2 Results and Discussion

6.2.1 Current Performance of brands in the on-trade

Initially the performance of two Molson Coors brands, Carling and Coors Light were benchmarked in the on-trade (Table 6.1). Carling is the biggest beer brand by volume in the U.K. selling over 3 million hL in 2018 (Morning Advertiser, 2018) and, accordingly throughputs of the brand would be anticipated to maintain quality.

Table 6.1 Carling and Coors Light on-trade performance.

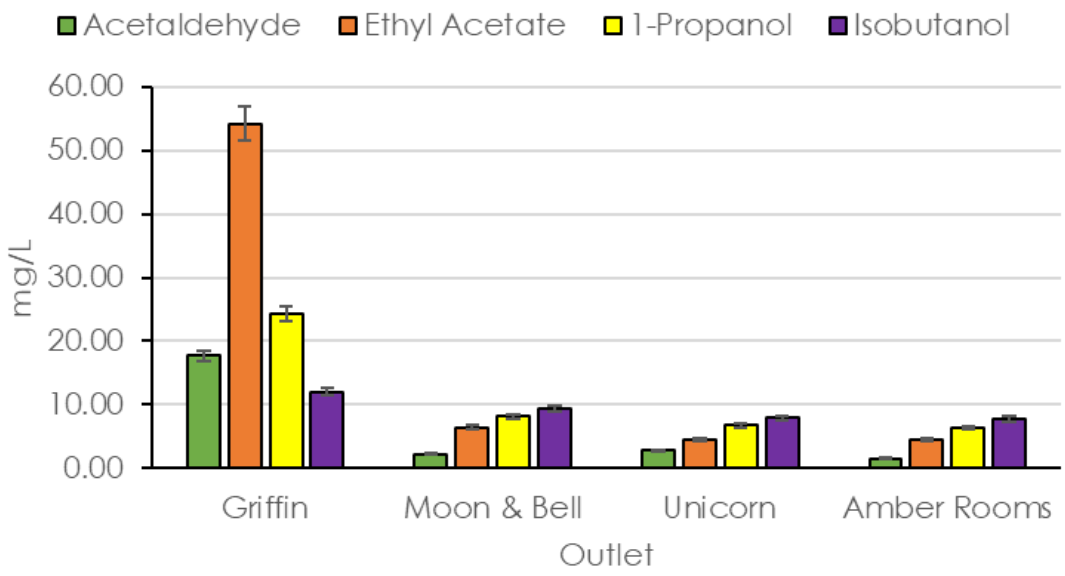
Brand	**Carling**	**Coors Light**
No. A (Excellent)	18	8
No. B (Acceptable)	10	10
No. C (Poor)	2	9
No. D (Unacceptable)	0	0
Quality Index (%)	88.3	74.1
Min OD_{660nm}	0.085	0.018
Max OD_{660nm}	0.808	0.758

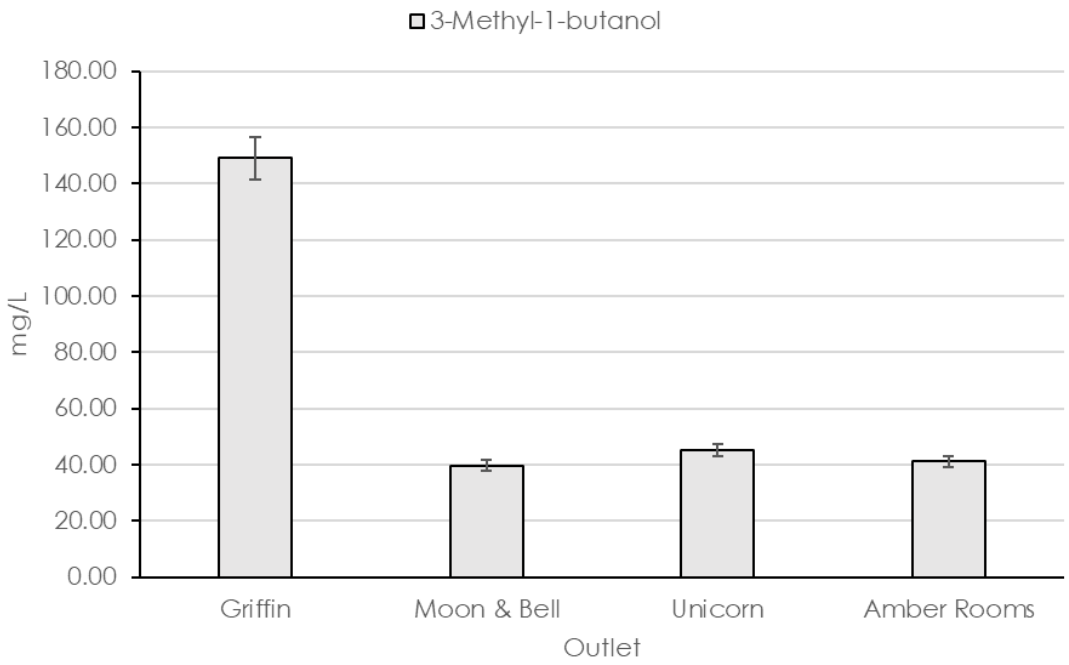
Mean OD_{660nm}	0.284	0.429
T-test p-value	0.017	

A total of ten accounts were visited of which, nine had both brands on the bar. The outlet removed Coors Light after the first sampling, so the result was removed. There is a significant difference (P=0.017) in the quality of the two brands in the on-trade with Carling performing better with 60% of samples in the excellent band (forcing test, Section 2.2) compared with 29.6% of Coors Light samples.

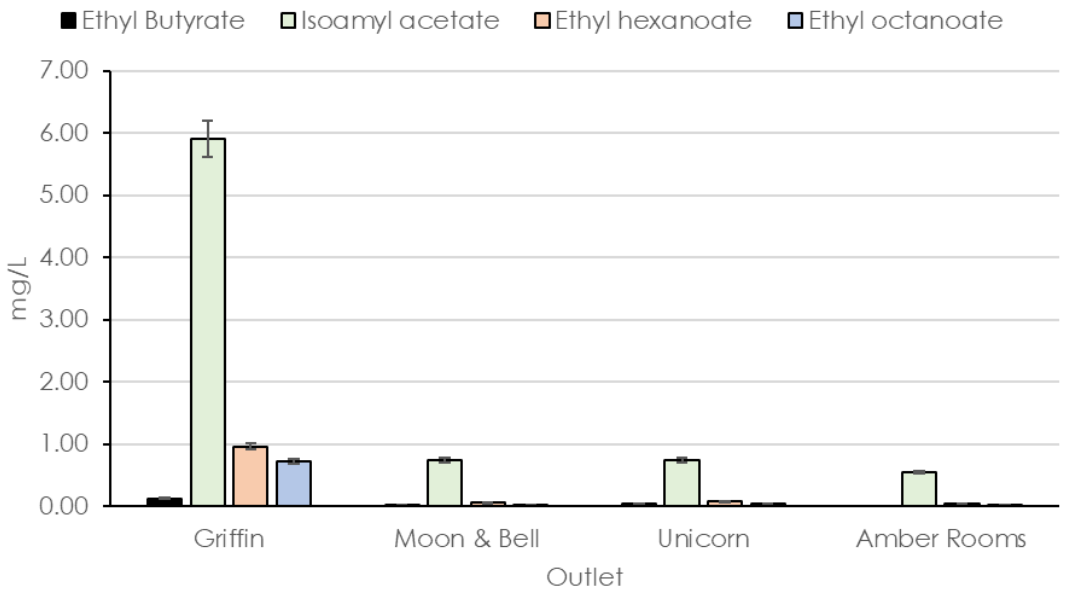
The profile of volatile compounds (Figure 6.1 A, B, C) and vicinal diketones (VDK) or diacetyl in Carling from four accounts in Loughborough (Figure 6.1D). One account, The Griffin, had significantly higher levels of the acetate esters, ethyl acetate and isoamyl acetate. These esters are secondary metabolites produced by yeast in fermentation, their structure consists of an acid group – acetate and alcohol group – ethanol. Small changes in the concentration of these compounds can have a notable effect on the sensorial quality (Cristiani and Monnet, 2001; Verstrepen *et al.*, 2003; Saerens *et al.*, 2008). The level of 3-methyl-1-butanol traditionally known as isoamyl alcohol was also significantly higher, this fusel alcohol has a flavour threshold of 70 mg/L (Ingledew, 1979) and can be produced by the Ehrlich

pathway in *Saccharomyces cerevisiae* (Hazelwood *et al.*, 2008) or by contaminating bacteria such as *Obesumbacterium proteus* (Ingledew, 1979).

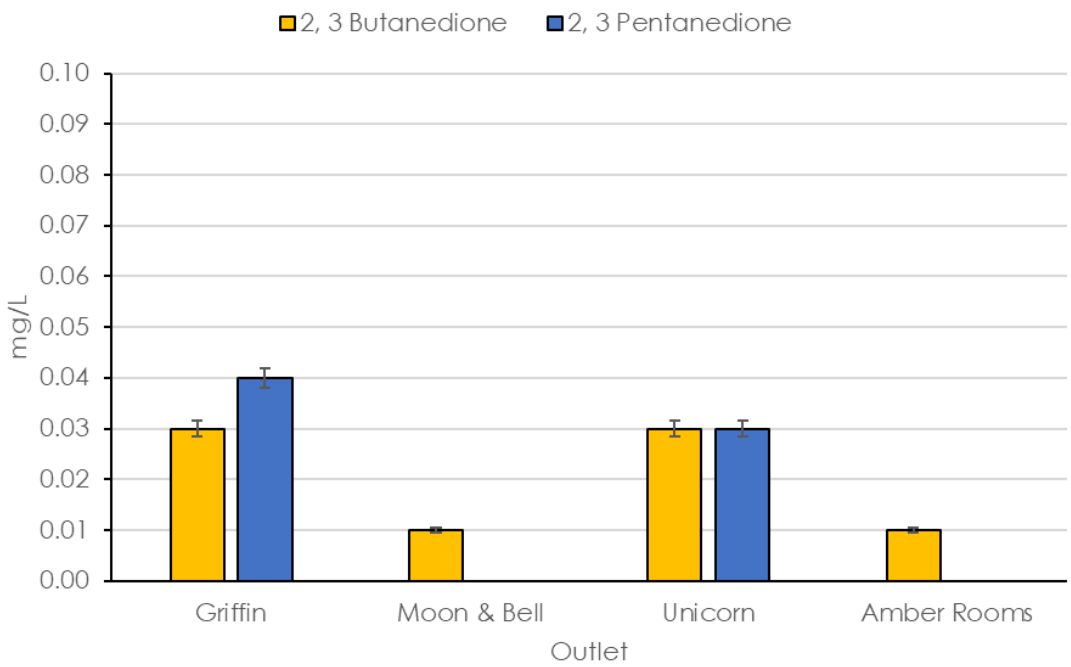



3-Methyl-1-butanol
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
mg/L
Griffin
Moon & Bell
Unicorn
Amber Rooms
Outlet

4017


Ethyl Butyrate
Isoamyl acetate
Ethyl hexanoate
Ethyl octanoate
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
mg/L
Griffin
Moon & Bell
Unicorn
Amber Rooms
Outlet

4018



4019

4020 **Figure 6.1**: Beer volatiles and vicinal diketone levels in Carling

4021 dispensed in four on-trade accounts.

This volatile data agreed with the forcing test as the Griffin performed worst of the four accounts with a mean OD_{660nm} of 0.465 whilst the three other accounts had mean OD_{660nm} values of 0.103, 0.155 and 0.109.

6.2.2 Validation of extended line cleaning frequency

6.2.2.1 Spoilage of new dispense system

In the development of the new dispense system, Molson Coors considered whether (or not) to copy the Heineken model and send out their own technicians to clean the lines. Clearly, this comes at a cost which can be maintaining quality for an extended period. Accordingly, the initial target was reduction from weekly cleaning to every four weeks.

Initially, the newly installed dispense system was filled with spoilt beer from the on-trade, the on-trade samples used in section 6.2.1 were pooled together and forced with fresh Carling used as the growth media (see section 2.5.2.1).

All lines scored in the poor or unacceptable category after the system was filled with spoilt beer. Remote coolers were turned on after seven days and the line volume of beer (2500 mL) dispensed every three days for two weeks to ensure fresh media was pulled into the lines to allow for microbial growth. The quality of the beer improved slightly once the regular dispense of the line volume was initiated, with presumably, the turbulence of the flow was

disrupting any loosely attached biofilm in the line. Prior to the first line clean all lines were in the poor or unacceptable quality band.

Differential centrifugation was used to assess the composition of the planktonic microorganisms in each line throughout the 'spoilage phase'. These results offered an early indication of the propensity for different conditions to encourage the growth of a different microflora. Table 6.2 shows the yeast and bacteria (as a percentage) split using low-speed differential centrifugation with forced samples.

Table 6.2 Microflora composition between line materials during spoilage phase.

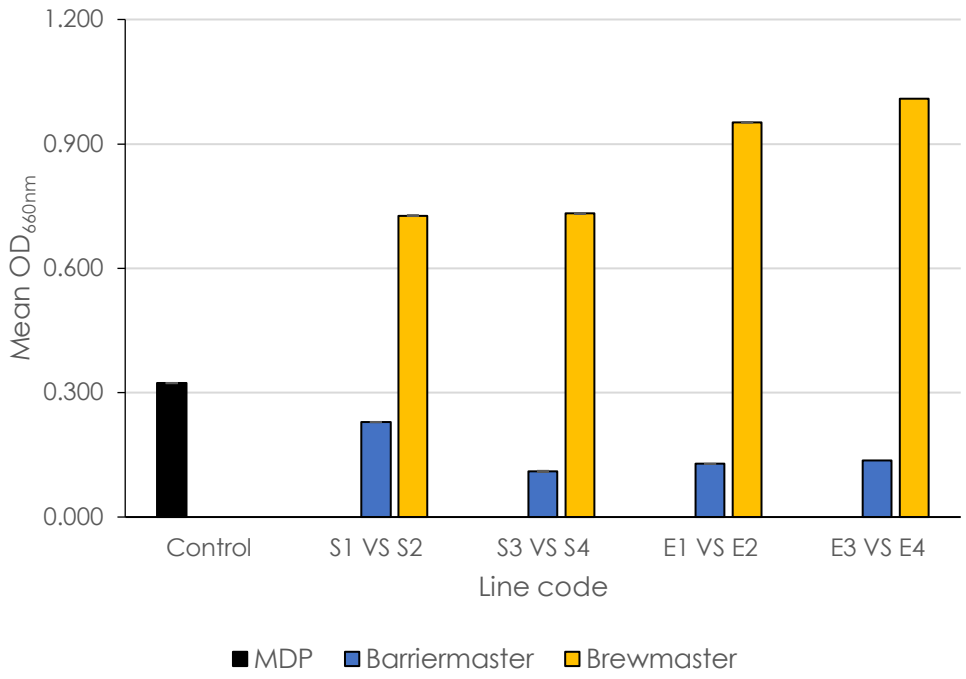
Line Material	**Presumptive yeast or bacteria**	**Day 7 (%)**	**Day 14 (%)**	**Day 21 (%)**
MDP	Yeast	76.7	81.7	74.0
	Bacteria	23.3	18.3	26.0
Barriermaster Flavourlock (FL)	Yeast	72.2	75.7	76.5
	Bacteria	27.8	24.3	23.5
Brewmaster 2 (BM)	Yeast	91.0	87.2	76.2
	Bacteria	9.0	12.8	23.8

As can be seen in Table 6.2 the microflora composition was stable in the Barriermaster Flavourlock (FL) lines, with approximately one quarter of the population (as absorbance) contributed by

bacteria across the three sample points. Conversely the Brewmaster 2 lines which allow greater oxygen ingress (Table 5.7) were initially supportive of a higher % yeast growth. The composition then changed over a 14-day period to match that of the FL lines.

This suggests aerotolerant bacteria such as the LAB were able to grow well in the FL lines straight away whereas the conditions needed to change to support bacterial growth in the BM lines, these changes may have been caused by the yeast enabling the growth of the bacterial contaminants (Vaughan et al , 2005).

Furthermore, the Brewmaster lines with presumably higher levels of dissolved oxygen, developed more biofilm during the spoilage phase (Figure 6.2). Yeasts are the dominant organisms in brewery biofilms (Timke *et al.*, 2008) and these lines would also support the growth of Gram negative acetic acid bacteria which alongside yeasts were seen to be the initial colonisers of brewery plant by (Storgårds *et al.*, 2006). The action of these initial colonisers in reducing pH in the presence of oxygen then enables growth of facultatively aerobic slime producing bacteria (Maifreni *et al.*, 2015).



4080

4081 **Figure 6.2**: Biofilm level for each line after the initial spoilage phase
4082 on a newly installed dispense system.

4083 ***6.2.2.2 Beer quality ex dispense 7, 28 and 42 days***

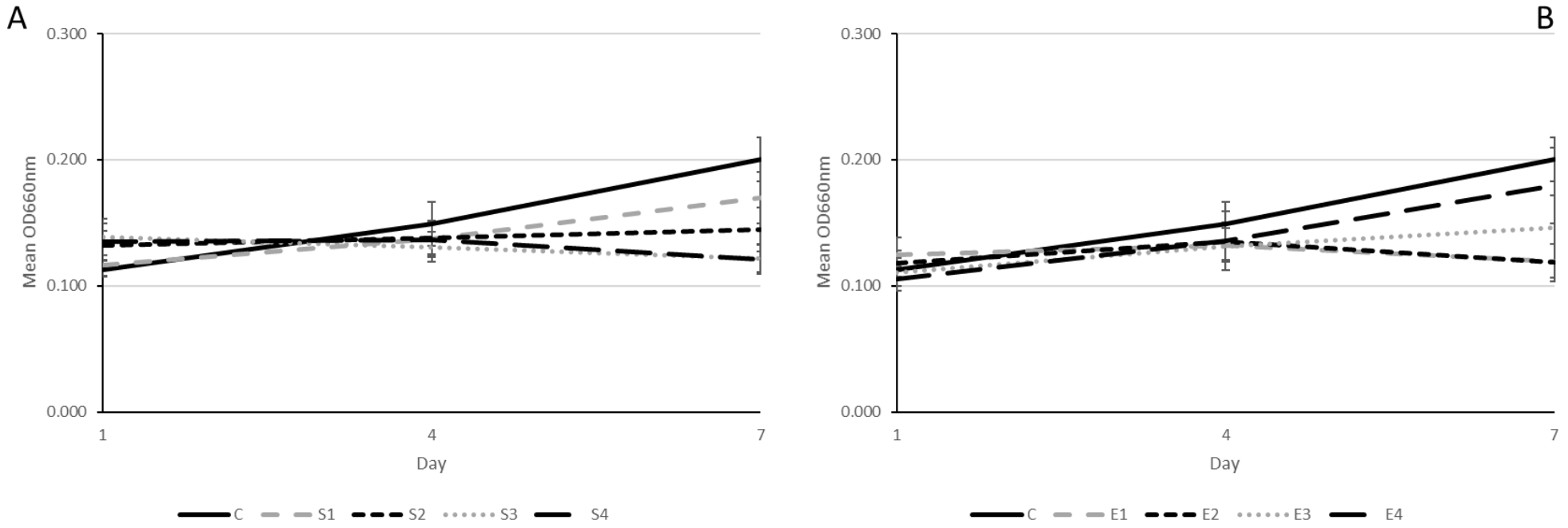
4084 Each week, a fresh keg of beer (Carling) was attached and
4085 dispensed daily at a similar rate to on-trade throughputs (section
4086 2.5.3). Beer quality at the point of dispense was measured on day
4087 1, 4 and 7 and this was every week for both the four weekly cycle
4088 and six weekly cycles.

4089 Figure 6.3a shows the mean beer quality across the standard
4090 temperature (6-8°C) lines and Figure 6.3b shows the results for extra
4091 cold (1-4°C). All lines were consistently 'excellent' from day 1
4092 through day 7 and there were no significant differences regardless
4093 of dispense temperature, line material or font type. Accordingly,

the cleaning frequency can be confidently extended beyond the recommended 7 days when the throughput is equivalent to 50 L or greater per week. Of the various lines, the worst performing line by the end of the week was the control line (equivalent to current dispense systems in place across the U.K) but despite this was still well within the excellent quality band (OD_{660} 0-0.300).

Following the successful validation of the 7-day cycle, the target of 28 days (4 weeks) was tested. Again, a new keg was connected every 7 days and 50 L dispensed across each 7-day period to replicate on-trade throughputs.

Figures 6.4 and 6.5 shown the mean beer quality ex-dispense achieved across the 28-day period between cleans (three periods combined). For the entirety of the 28-day period, the results remained within the excellent (0-0.300) and acceptable (0.301-0.600) quality bands. Only line E1 (Barriermaster Flavourlock, extra cold dispense temperature and cooled font) exhibited a significantly better beer quality than the control line across the 28-day period, with a p-value of 0.04.



4112

4113 **Figure 6.3**: Mean across a 7-day period between line cleaning, at standard dispense temperature 6-8°C (A) and extra cold

4114 dispense temperature 1-4°C (B). Samples taken for 3 x 7-day periods with error bars showing standard error.

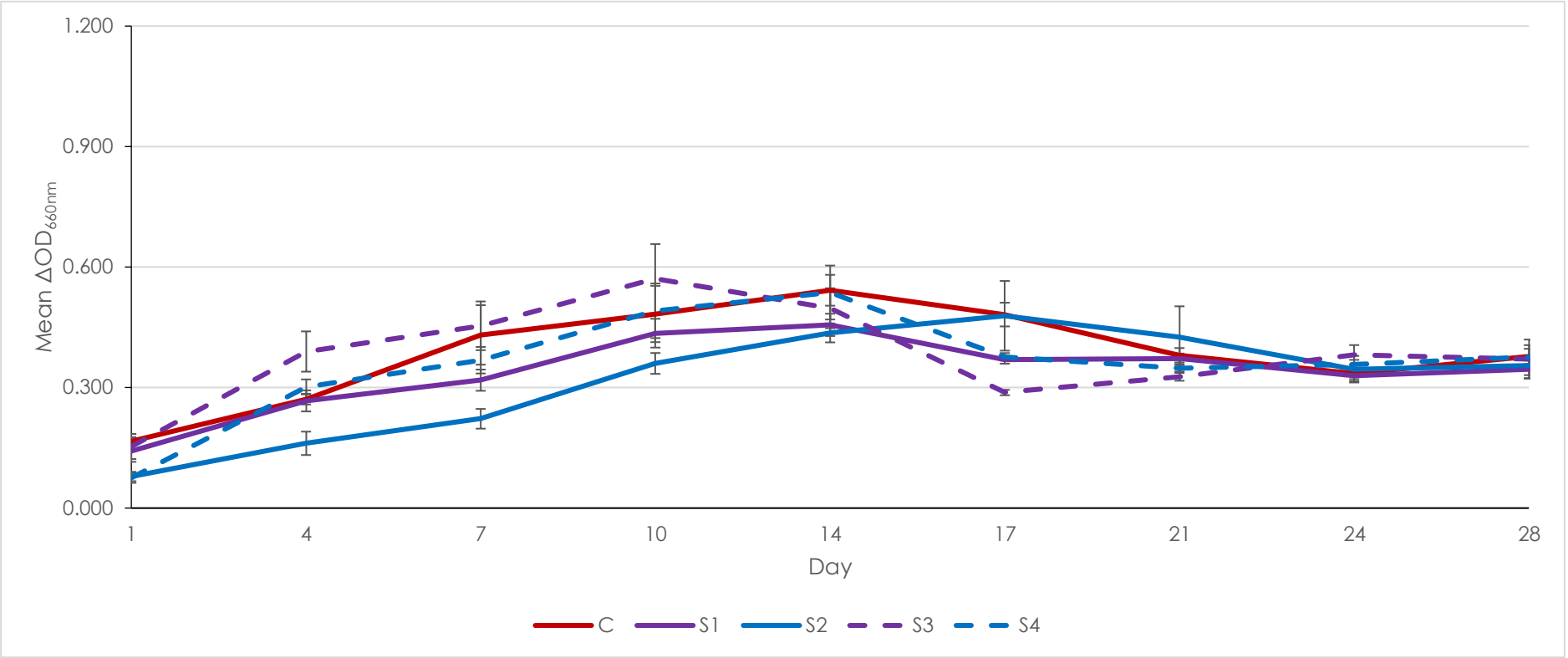


Figure 6.4: Mean beer quality ex-dispense at 6-8°C dispense temperature, across 28-day period. Three periods combined with standard error shown by error bars.

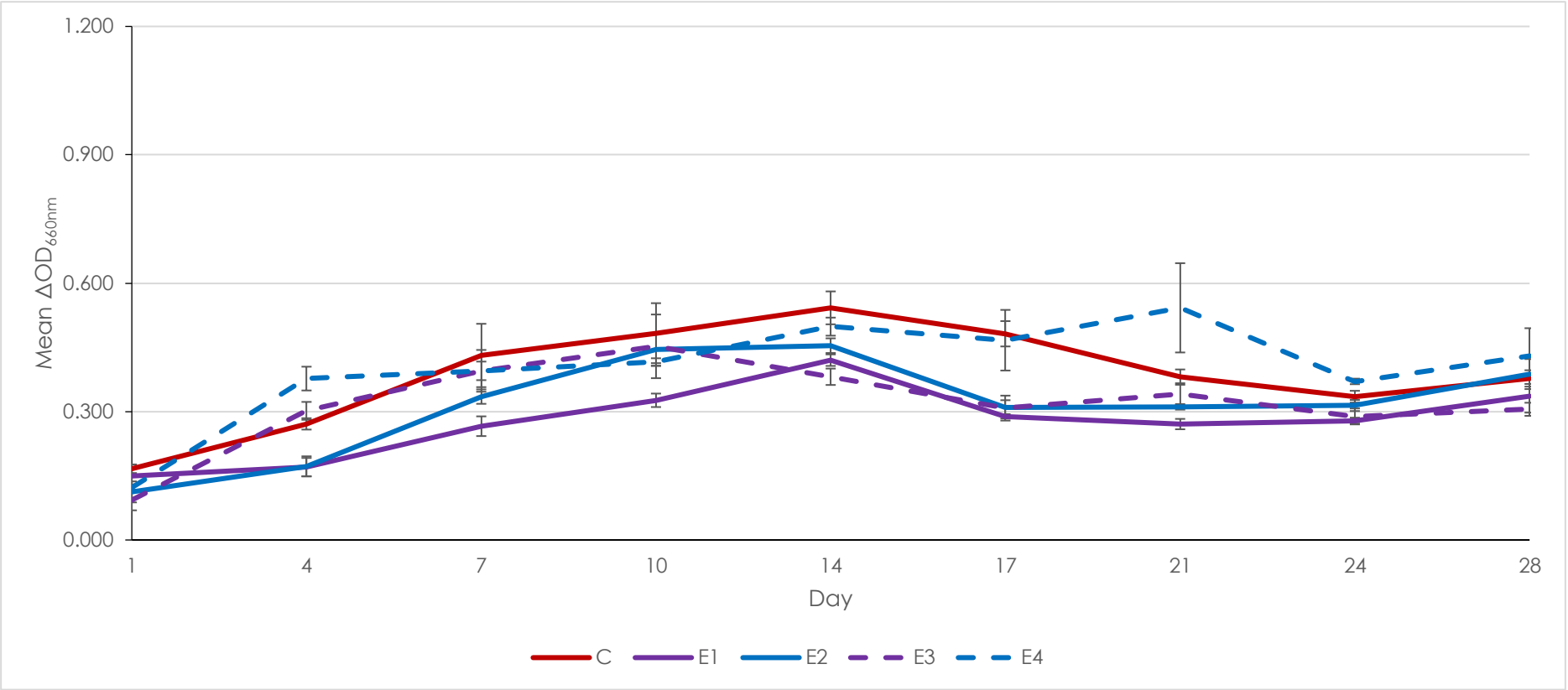


Figure 6.5: Mean beer quality ex-dispense at 1-4°C dispense temperature, across a 28-day period. Three periods combined with standard error shown by error bars.

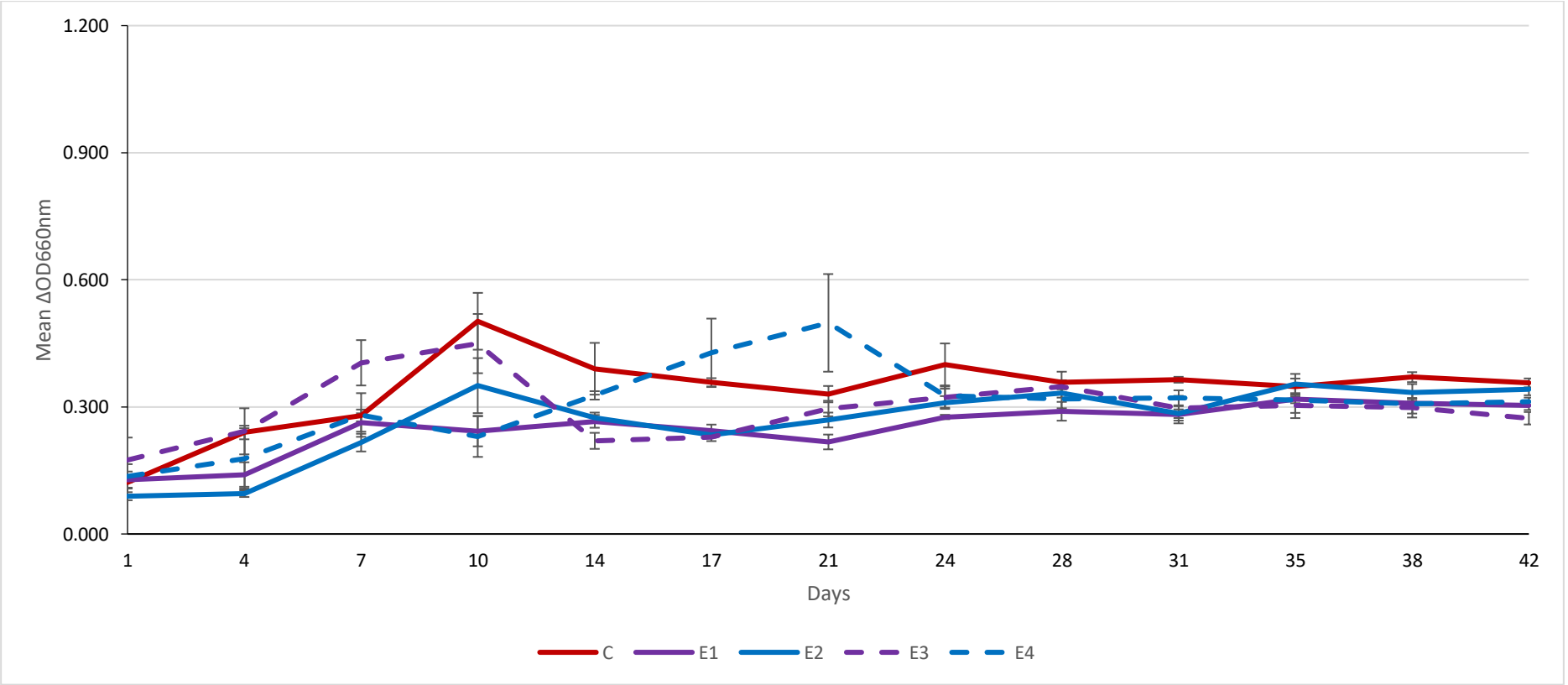
Indeed, the mean quality scores for the beer from line E1 were in the excellent band (66.6%) with the other 33.3% being just inside the acceptable band. The performance over 28 days, of the Barriermaster Flavourlock line at extra cold temperature with a chilled font, suggests that quality in glass would be suitable for consumers as long as throughput is at least 50 L/week.

As none of the standard temperature lines performed significantly better than the control line, this temperature range was 'parked' as a contender for an extension of line cleaning frequency. However, a further 18 weeks of testing was performed on the extra cold lines with control line for comparison. The lines were cleaned every six weeks (42 days) and quality in glass measured periodically across each six-week period. The mean scores for each line can be seen in Figure 6.6.

Again, line E1 was of significantly ($p<0.01$) better quality than the control line as was also line E2 (Extra cold temperature with Brewmaster 2 line type) ($p<0.05$). Table 6.3 shows an overview of the quality scores achieved by lines C, E1 and E2.

Table 6.3 Quality (as OD_{660}) for lines C, E1 and E2 for 3 x 42-day trial.

Line code	Mean	Min	Max	Range
C	0.340	0.122	0.502	0.380
E1	0.252	0.129	0.318	0.190
E2	0.268	0.089	0.354	0.265



4141

4142 **Figure 6.6**: Mean beer quality ex-dispense at 1-4°C (Extra Cold) dispense temperature, for 42-day period. Mean of three cycles

4143 of 42 days with standard error shown by error bars.

Beers from line E1 were 'excellent' for 76.9% of samples compared to 53.8% for line E2 and 23.1% for the control line (C). With Line E1 peaking just inside the acceptable band at 0.318 and not until the final week (six). It is suggested that beer quality from line E1 would be sufficiently robust to prolong the period between line cleans to 42 days (6 weeks) with the caveat of a throughput of at least 50 L/week.

6.2.2.3 Measurement of line biofilm after 7, 28 and 42 days

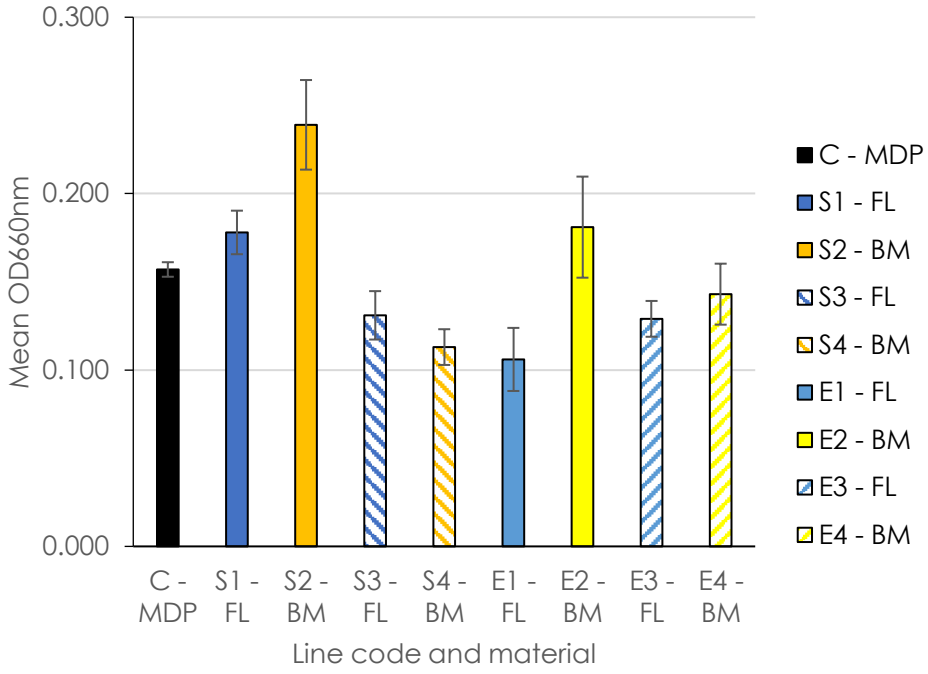


Figure 6.7: Recovered biofilm loading from dispense lines pre-cleaning after seven day dispense cycle between line cleaning. Three x seven-day periods measured with standard error shown by error bars.

As was noted in the results from the spoiled lines prior to initial line cleaning trials, levels of recovered biofilm (Figure 6.7) are higher in the Brewmaster 2 lines than their Flavourlock counterparts except

for line S4, which is the Brewmaster 2 line at 6-8 ° C, dispense temperature. The atmosphere within the Brewmaster line is presumably more hospitable to initial biofilm colonisers (aerobic microorganisms) whether they be environmental contaminants from the tap end or recovering beer spoilage bacteria not removed by cleaning (Storgårds *et al.*, 2006).

To enable a comparison, the same OD_{660nm} bands were used to plot data for the biofilm (Figure 6.7) and it can be seen that each line remains within the excellent quality band for the 7-day period, supporting the beer quality ex-dispense results seen in Figure 6.3.

As the beer quality in glass for the standard temperature dispense systems during the 28-day cleaning frequency trials were not significantly better than the control line, biofilm level was measured at 4 and 6 weeks in the control and extra cold lines only. Figure 6.8 shows the mean biofilm recovered at the end of the 1, 4- and 6-week cycles between cleans.

The level of biofilm recovered from line material Brewmaster and Barriermaster Flavourlock is much less than that from the control (MDP) line. However, the extent of difference increases as the line cleaning period is extended. The Barriermaster Flavourlock lines show lower biofilm than Brewmaster at 1 week but then become comparable to Brewmaster at both 4 and 6 weeks, the inherent nature of the lines will change and as mentioned in 6.2.2 the initial

microbial colonisers will find Brewmaster lines more hospitable. The microorganisms in the Barriermaster Flavourlock lines initially do not form biofilm as effectively until they have adjusted or created the conditions suitable for their growth and attachment.

It is clear than current standard dispense systems in the U.K. using MDP as the line material are vulnerable to significant biofilm formation by extending the line cleaning period from 7 to 28 days or longer.

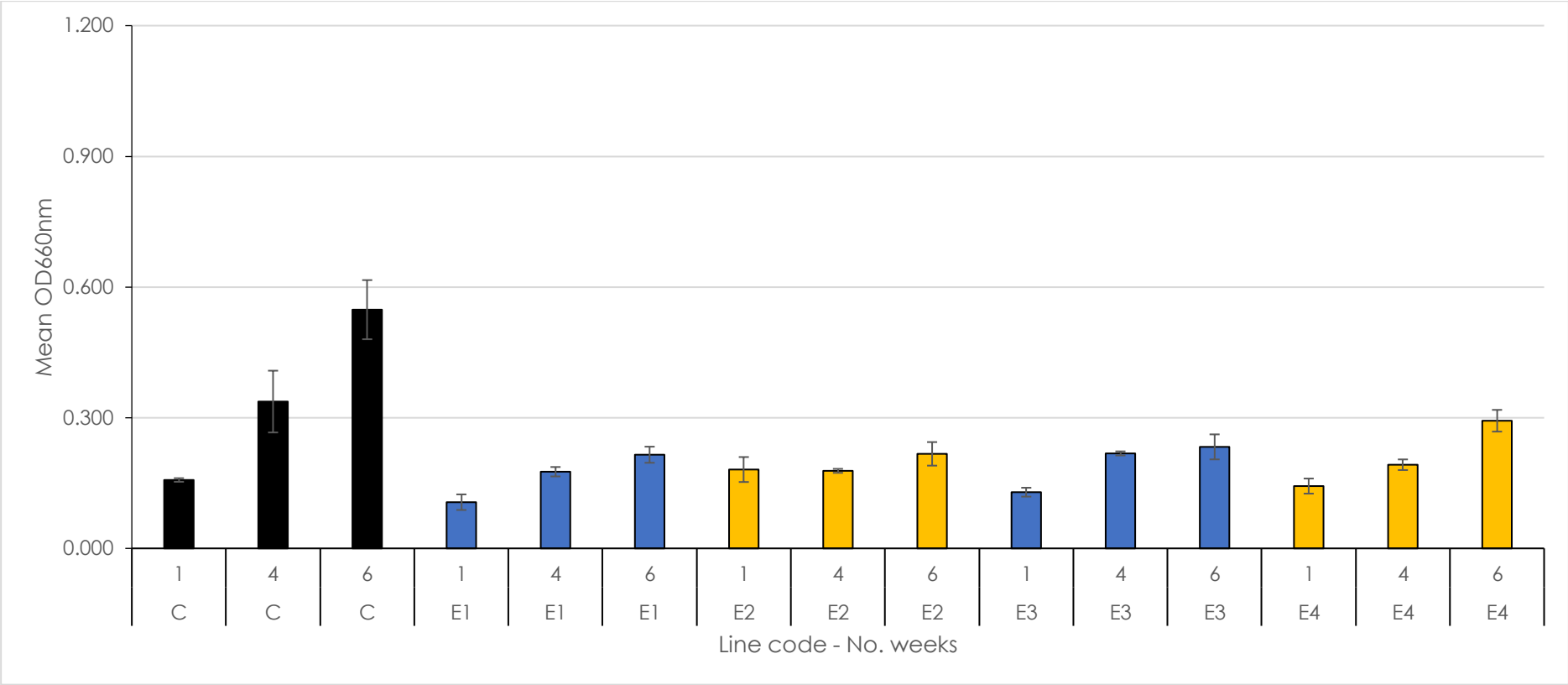


Figure 6.8: Mean biofilm level measured at 1, 4 and 6 weeks for control and extra cold lines only. C (**MDP**), E1 and E3 (Barriermaster Flavourlock) and E2 and E4 (Brewmaster 2). Mean of 3 x cycles with standard error shown by error bars.

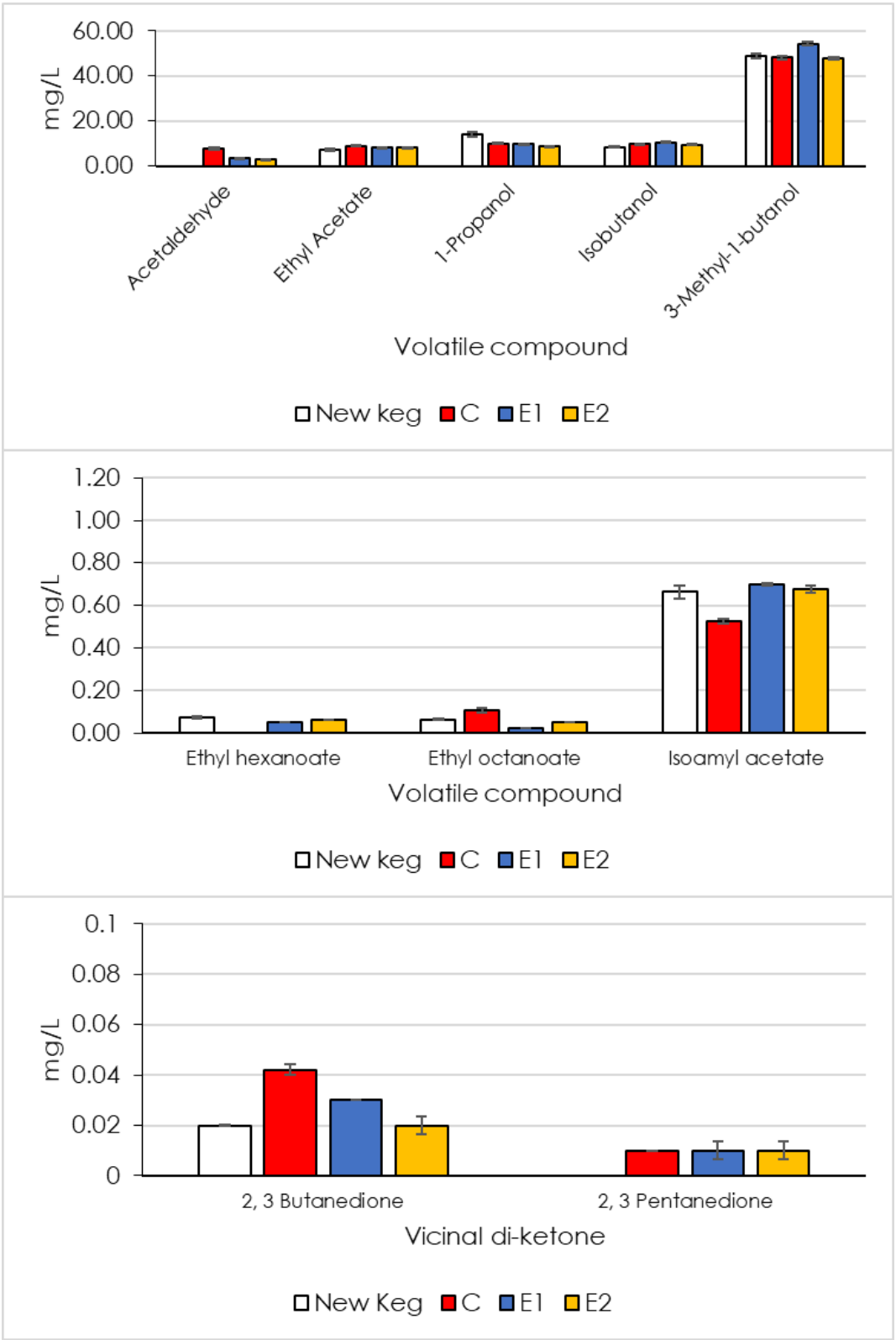
6.2.2.4 Volatiles and vicinal diketones (diacetyl) in beer ex dispense at 7, 28 and 42 days

Beer quality was also assessed by measurement of the volatile compounds and vicinal diketones found in beer ex-dispense. Initially, beer dispensed on day 7 was compared to that of a fresh sample from an un-broached keg (Figure 6.9). The only significant difference was in acetaldehyde which was not present in the 'new keg' samples but it was found in each of the three lines tested (C, E1 and E2). These lines were chosen because of their superior results in both beer quality ex-dispense and in recovered total biofilm.

Acetaldehyde is formed by the oxidation of ethanol so lines with higher dissolved oxygen would be more likely to see development of acetaldehyde. The flavour threshold levels in lager style beers are ≈ 10mg/L (Briggs *et al.*, 2004), and the acetaldehyde levels in the beer after one weeks dispense were below the flavour threshold at 7.5 (control), 3.2 (E1) and 3.1 (E2) mg/L.

The concentration of 2,3-butanedione or diacetyl was higher in the control and E1 lines when compared to the new keg though not significantly and again was below the flavour threshold of 0.1-0.2 mg/L in lager beer (Wainwright, 1973; Meilgaard, 1975). It should be noted that these samples were not 1st runnings and were taken after dispense of the daily throughput (as per the trade throughput, Table 2.11). In summary, as long as trade accounts are

practicing best practice(s) in beer dispense then beer quality - as assessed by measurement of volatiles - is assured at 7 days.



4219

4220 **Figure 6.9**: Beer volatile(s) and vicinal diketone levels measured in

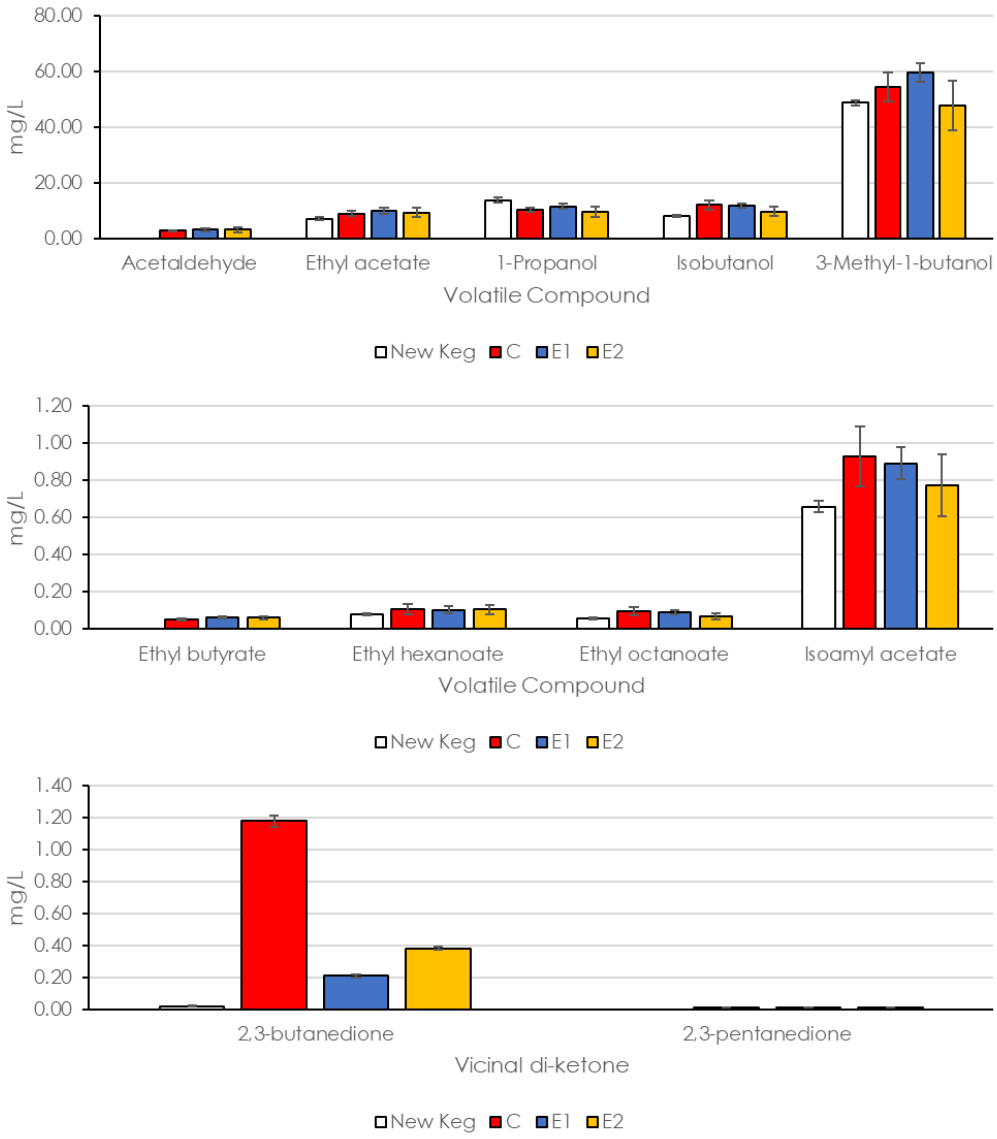
4221 beer at the end of seven days dispense compared to that of a fresh keg.

4222 Triplicate samples with standard error shown by error bars.

Further samples for volatile and VDK analysis were taken at 28 and 42 days (Figures 6.10 and 6.11). Again, as the best performance for beer quality ex dispense and for biofilm recovery at extended line cleaning frequencies with lines E1 and E2, only data for these lines is reported here compared to the new keg and the control line.

At 28 days, very low levels (≤ 0.06 mg/L) of ethyl butyrate were detected. Ethyl butyrate - also known as ethyl butanoate or butyric ether - is an ester providing a pineapple or mango type fruity flavour. It is readily produced as a yeast secondary metabolite and there are increased levels in aged beer (Lermusieau et al, 2001; Gijs *et al.*, 2002). It can also be formed by the esterification of butyric acid produced by bacterial contaminants and has a flavour threshold of 0.4 mg/L (Saerens *et al.*, 2008).

There was also a higher level of isoamyl acetate detected in all lines relative to that in the new keg. The difference was not significant for any of the lines however, isoamyl acetate is a commonly found ester in beer providing a pear drop flavour with a low flavour threshold of 0.2 mg/L (Peddie, 1990). As this low flavour threshold may mean that small differences in concentration are detected by a sensory panel, it is suggested this work is followed by a rigorous sensory study with a trained tasting panel.



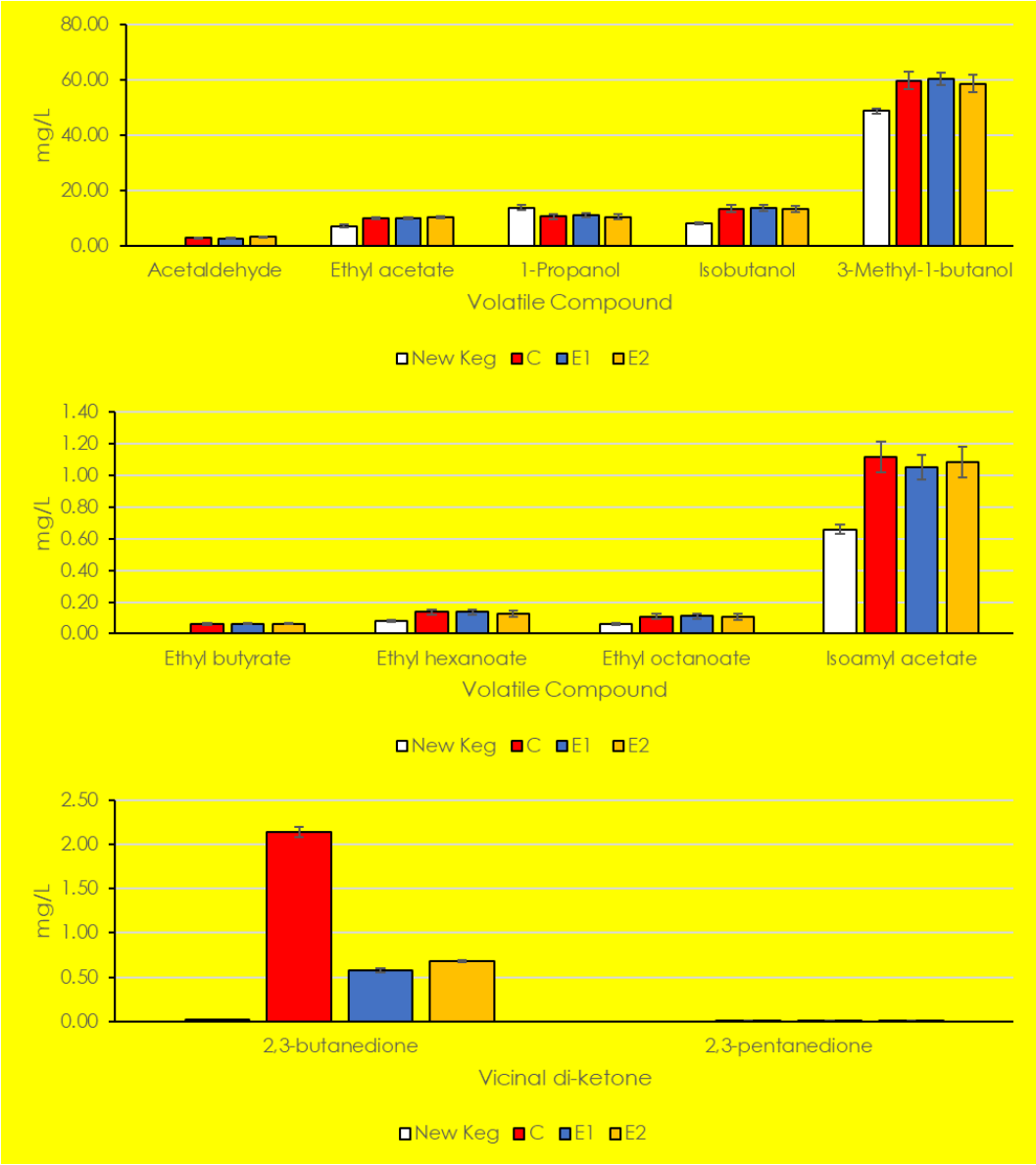
4245

4246 **Figure 6.10**: Beer volatile(s) and vicinal diketone levels measured in
4247 beer at the end of 28 days dispense compared to that of a fresh keg.
4248 Triplicate samples with standard error shown by error bars.

4249 Finally, a much higher level of 2,3-butanedione (diacetyl) was
4250 detected in the control line, this level (1.18 mg/L) is far higher than
4251 that after 7 days and is substantially higher than the flavour
4252 threshold for lager beers. Indeed, this level of diacetyl would
4253 render the beer unacceptable (Wainwright, 1973). The levels seen

in lines E1 and E2 are also higher than those recorded after 7 days. However, for line E1 the value of 0.2 mg/L is borderline with regard to the flavour threshold and it is therefore suggested this would be an acceptable level in dispensed beer.

At 42 days, the results mirror that of 28 days with only a slight increase in isoamyl acetate and 3 methyl-1-butanol. The differences in the volatile compounds are not significant when compared with those detected at 28 days or from a new keg. That does not mean however, that these relatively small increases in compounds are undetectable by consumers. The biggest issue affecting beer quality at 42 days is the level of vicinal diketones. The level of diacetyl detected in the control line is almost twice that at 28 days and a level of 2.15 mg/L is 10x the flavour threshold in lager beers (Wainwright, 1973; Krogerus and Gibson, 2013). Furthermore, the levels detected in E1 and E2 are 0.58 and 0.68 mg/L respectively, which would be unacceptable in Carling. Therefore, an extension to a six weekly line cleaning frequency would leave Carling vulnerable to a high loading of diacetyl which would have a detrimental effect on beer quality. The relatively small changes in volatile compounds seen at 4 and 6 weeks compared to the new keg levels are surprising however it may be explained by the fact that each keg is dispensed for 7 days due to the throughput levels used to mirror on-trade conditions.



4277

4278 **Figure 6.11**: Beer volatile(s) and vicinal diketone levels measured in
4279 beer after 42 days dispense compared to a fresh keg. Triplicate
4280 samples with standard error shown by error bars.

4281 Again, suggesting that rate of throughput is critical to dispense
4282 beer quality (section 5.2.3), as all the above results are only
4283 applicable and validated against a throughput of 50 L per week.

6.2.2.5 Comparison of font cooling

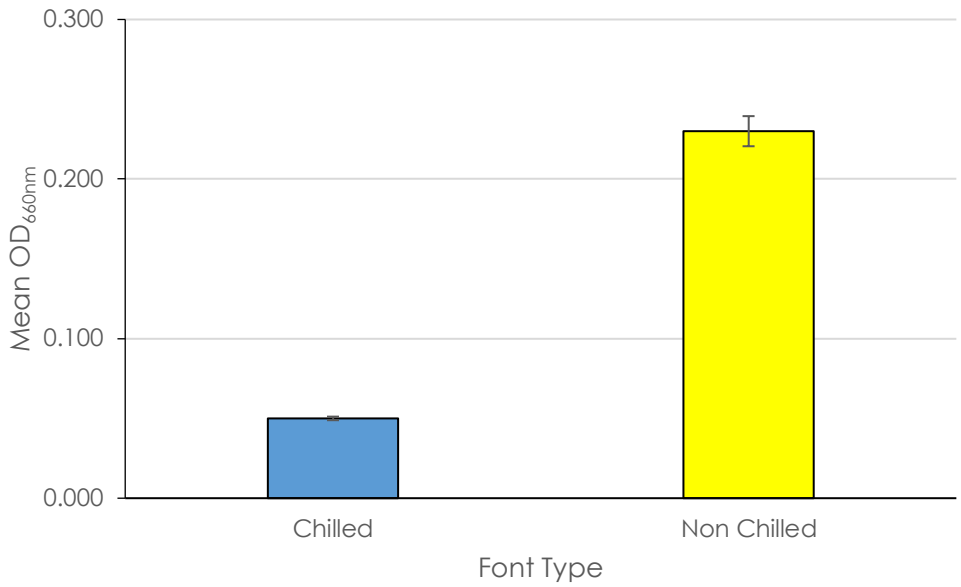
Finally, the impact of additional cooling within the font body was measured. The tap was disconnected from the font body and the rim at the font where the tap is connected swabbed for biofilm recovery (Figure 6.12 and 6.13). It can be observed that there is less biological material on the chilled font (on the right) compared to the non-chilled font (on the left) and this is supported by the data.

Building in protection for the tap is a sensible strategy for improving beer dispense, as the tap is sat at ambient temperature and is an entry point into the beer dispense system. This is accessible to environmental organisms, which may be initial colonisers for biofilm development and facilitate the growth of beer spoilage microorganisms resulting in spoilt beer.

In accounts where food is also served by bar staff there is the risk – in the absence of proper hygienic practices - of cross contamination of food pathogens to the tap body when handling tap nozzles. This threat is exacerbated by poor personal hygiene of bar staff.



Figure 6.12: Images taken of the font body where the tap is connected to the font. The left-hand images shown are of non-chilled fonts and show visible growth of microorganisms. The right-hand images show a chilled font where little or no visible growth can be observed. Images were taken on the same day as the same time in the cleaning cycle.



4306

4307 **Figure 6.13**: Mean OD of swabs used to recover microorganisms from
4308 the font body. Mean of three swabbing cycles with standard error
4309 shown.

4310 ### 6.2.3 Trial 2: Comparison of the efficacy of four different
4311 line cleaning solutions

4312 This experiment was performed for Molson Coors to understand the
4313 best line cleaner for the new dispense system. This was performed
4314 with the four original standard temperature dispense lines but at
4315 extra cold dispense temperatures., The lines were re-fitted with
4316 similar fonts (non-cooled) so as to make two pairs of lines. Each line
4317 could then be cleaned with two of the four trial cleaning
4318 chemicals for three weeks and then tested with the other two of
4319 four cleaning chemicals for a further three weeks.

4320 To encourage fouling of the lines, keg throughput was reduced to
4321 25 L per week, with a single dispense period each day. This was

anticipated to have a detrimental impact on beer quality post cleaning due to keg contamination. Figure 6.14 shows the effect of reconnection of previously used kegs post cleaning compared to that of fresh kegs.

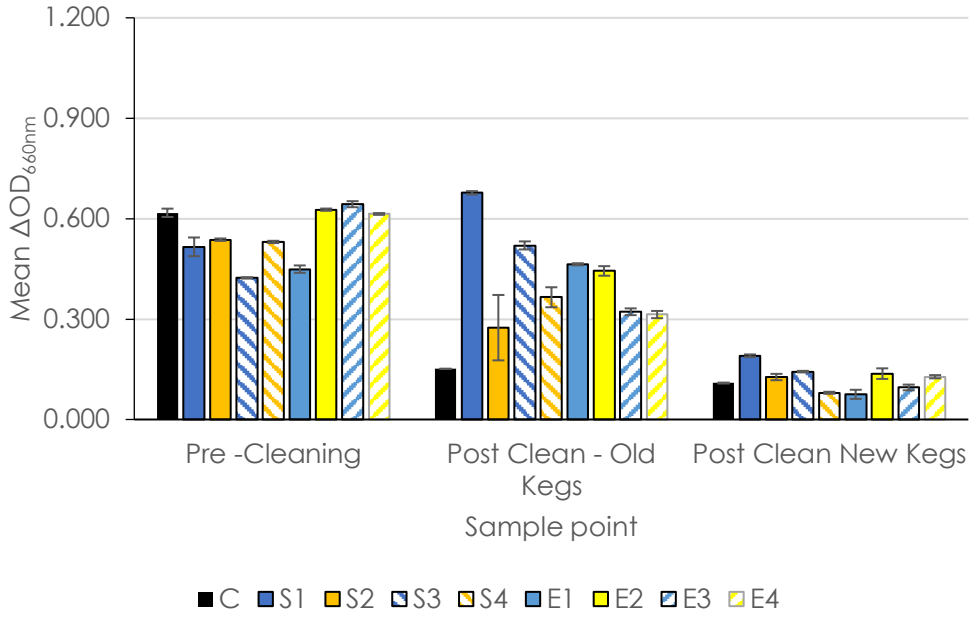


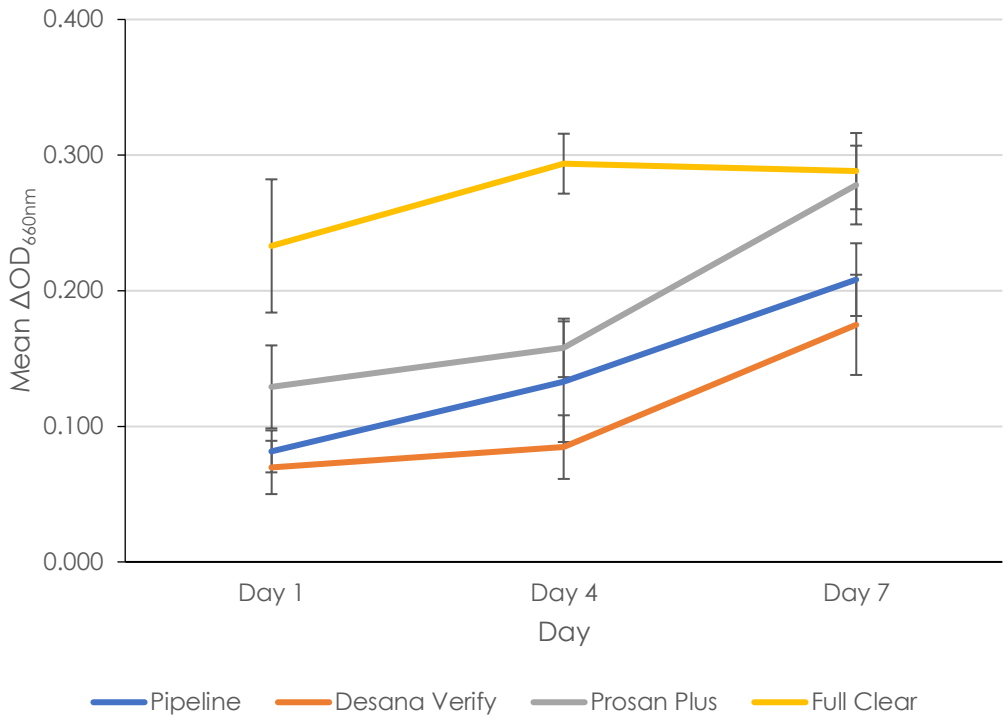
Figure 6.14: Mean ΔOD_{660nm} for beer quality ex dispense prior to cleaning and post cleaning with re-attached old kegs and then attachment of fresh kegs. Triplicate samples with standard error shown.

It can be clearly observed that used kegs are contaminated with varying levels of microorganisms, the re-attachment of these kegs to a cleaned dispense line would encourage quicker re-colonisation of beer lines and negatively affect beer quality post cleaning.

6.2.3.1 Beer quality ex dispense

Throughout the line cleaning trial, beer quality ex dispense was measured after cleaning (Day 1), during the week (Day 4) and before cleaning (Day 7). The line cleaner 'Full Clear' is designed (according to the manufacturers guidelines) to be used every four weeks therefore the results were not included in any statistical tests for direct comparison to the other three line cleaning solution 'Pipeline Original', 'Desana Verify' or 'Prosan Plus'.

With beer lines constructed of Barriermaster Flavourlock line (Figure 6.15) both Pipeline Original and Desana Verify performed similarly to each other with both products giving significantly better beer quality results for pre- and post-cleaning than Prosan Plus.



4350

4351 **Figure 6.15**: Mean beer quality ex dispense on Day 1 (post cleaning),
4352 Day 4 and Day 7 (pre-cleaning) of weekly cleaning cycle on
4353 Barriermaster Flavourlock lines. Beer throughputs were 25 L/week.
4354 Mean of 4 cycles, error bars show 95% confidence interval.

4355 However, this was not repeated using the Brewmaster 2 type of line
4356 (Figure 6.16). Desana Verify produced significantly better post
4357 cleaning results than Pipeline Original ($p=0.02$) and performed
4358 better than Prosan Plus. After seven days between cleans, Desana
4359 Verify and Pipeline Original lines exhibited the poorest beer quality.
4360 Here, beer from Prosan Plus cleaned lines was of significantly better
4361 quality at seven days.

4362 It should be noted that although Desana Verify cleaned lines were
4363 on average the poorest quality by day seven, they were the best

quality post cleaning. So, for cleaning efficacy, Desana Verify was the most effective line cleaner with Brewmaster 2 lines. With Barriermaster Flavourlock lines, Pipeline Original performs similarly to Desana Verify with the best beer quality after cleaning.

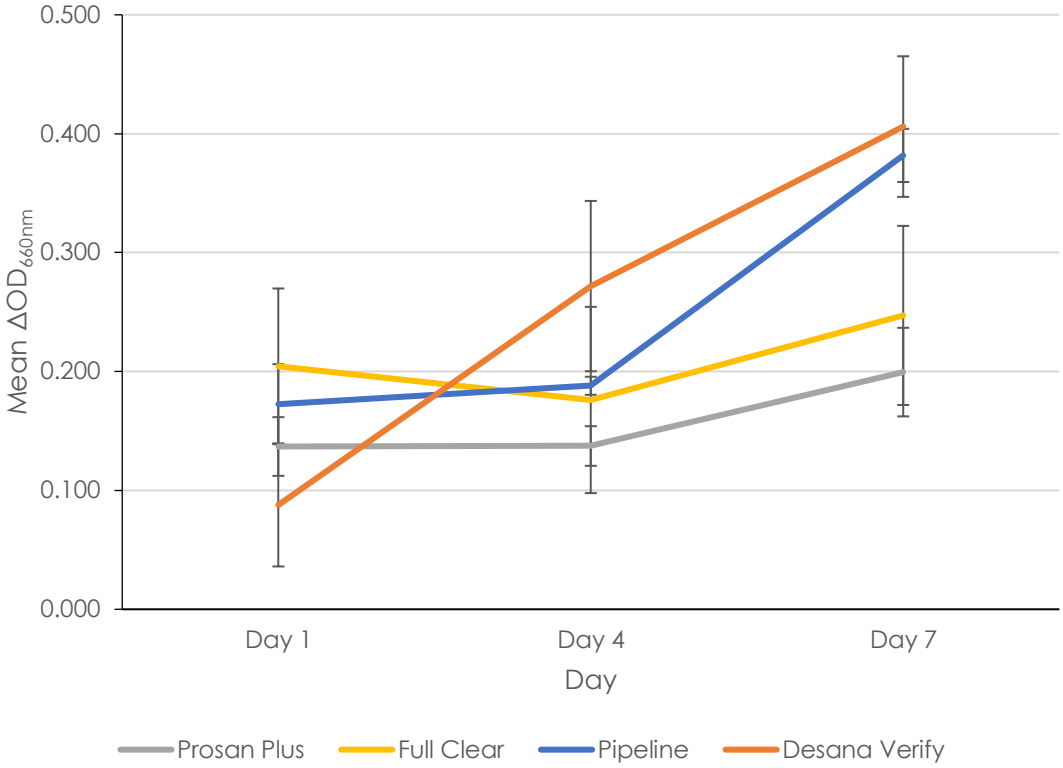


Figure 6.16: Mean beer quality ex dispense on Day 1 (post cleaning), Day 4 and Day 7 (pre-cleaning) of weekly cleaning cycle on Brewmaster 2 lines. Beer throughputs were 25 L/week. Mean of 4 cycles, error bars show 95% confidence interval.

The variation in performance of the two-line types is suggested to be because of the different gaseous environments within them. The Barriermaster Flavourlock line is better at maintaining levels of dissolved CO_2 and reducing the ingress of oxygen compared with

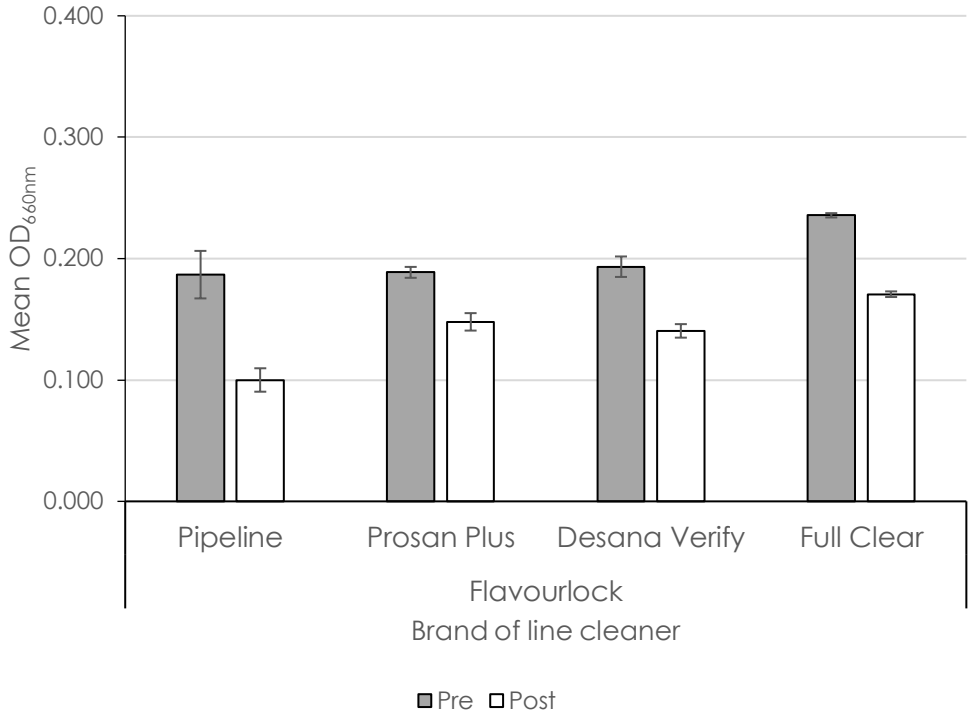
the Brewmaster 2 line which is an MDP line with a nylon coating to make the surface smoother and less suitable for microbial attachment.

It is therefore likely that the microflora capable of growth and spoilage of beer is variable within each of the line types, this is discussed further in Chapter 7 where an initial view of the microbiome of draught beer dispense is discussed with relation to these two-line types. Essentially, greater numbers of viable anaerobic cells were recovered from Barriermaster Flavourlock lines than were observed in the Brewmaster 2 lines whereas yeasts were the dominant microorganism recovered in the more aerobic Brewmaster 2 line.

6.2.3.2 Level of biofilm recovered pre- and post-cleaning

Alongside a consideration of the beer quality in glass, biofilm growth on the line surfaces was measured prior to and post cleaning. In accordance with the beer quality data, the levels of recovered biofilm were lowest in Barriermaster Flavourlock lines (Figure 6.17) for all line cleaning chemicals tested. The lowest biofilm levels were found when cleaning had been performed with either Pipeline Original or Desana Verify. Prosan Plus performed similarly on both line materials and the enzymic cleaning method employed by the Full Clear system showed some effect on

4399 Barriermaster Flavourlock but little or no effect on the Brewmaster
4400 2 lines (Figure 6.18).



4401

4402 **Figure 6.17**: Comparison of four-line cleaning chemicals on the mean
4403 biofilm levels recovered from Barriermaster Flavourlock dispense
4404 lines both pre- and post-cleaning. Results are the mean of 4 cleaning
4405 cycles with standard error shown.

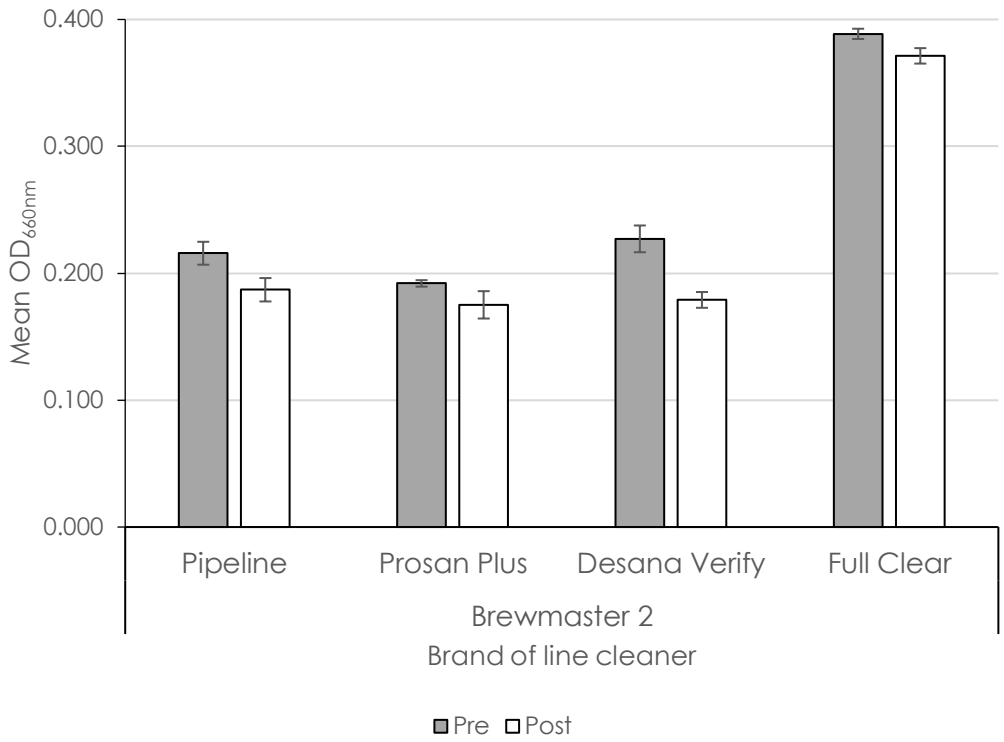


Figure 6.18: Comparison of four-line cleaning chemicals on mean biofilm levels recovered from Brewmaster 2 dispense lines both pre and post cleaning. Results are the mean of 4 cleaning cycles with standard error shown.

6.3 Conclusions

Work reported here, validates and supports the development of a novel dispense system designed by Molson Coors Brewing Company (MCBC). The main aim was to validate the new system and provide evidence for the use of particular dispense system components such as font type, line material and dispense temperature, and which of these assured beer quality better than the control system (designed to match current U.K. dispense

systems) and to then push the line cleaning frequency as far as was practicably possible without affecting beer quality.

This was done by performing many cleaning cycles, three each of 7, 28- and 42-day cycles representing 1, 4 and 6 weeks of dispense between cleans. Beer quality was measured in three ways; the forcing test was used to measure beer quality in glass i.e. what the consumer experiences, biofilm recovery from the surfaces of beer dispense lines was measured to see the propensity of a particular line type to support biofilm. It was considered that the more biofilm deposited the harder cleaning would become and with an increased frequency between cleans the biofilm would become a more mature and resistant biological structure. Finally, some of the common beer volatiles, mainly esters and higher alcohols, and the diacetyl were measured to understand the formation of potential off flavours and variation within the desired beer flavour profile.

The data shown here supports the use of an extra cold dispense temperature combined with the Barriermaster Flavourlock line material, beer quality in glass was significantly better with this combination than that achieved in the control system (MDP) and with the Brewmaster 2 lines. This was also true of biofilm formation, where Barriermaster Flavourlock lines showed the lowest propensity for biofilm formation compared with the other line materials at

both 4 and 6 weeks. Furthermore, there is evidence for the use of a chilled font as it reduces the growth of microorganisms with the font and tap body, protecting a key point of entry into the dispense system from ingress of environmental organisms.

The beer volatiles were consistent between all line materials and the extension of the line cleaning frequency had no significant effect on these levels although small increases were observed. However, the levels of the vicinal di-ketone, diacetyl (2,3-butanedione), were significantly higher in all line materials six weeks after cleaning. Barriermaster Flavourlock was the best performing line as the level of diacetyl was lowest however it was still three times the flavour threshold of diacetyl reported for lager style beers (Wainwright, 1973; Krogerus and Gibson, 2013).

For this reason, it is suggested that cleaning can only confidently be extended to four weeks without detriment to beer quality. Importantly the evidence reported here is only acceptable if the throughput of the line is at least 50 L/week i.e. one container per 7 days of trading at a minimum. Such throughputs however are a likely pre-requisite of installation of the MCBC dispense system.

The second phase of work on the Molson Coors dispense system was to conduct a comparison of four-line cleaning chemicals, three from established industry suppliers and a novel enzymic cleaner (Full Clear).

Each cleaner was assessed with both Barriermaster Flavourlock and Brewmaster 2, lines. Their comparative efficiency was measured by forcing (beer quality in glass) and measurement of biofilm on the line surface pre- and post-cleaning.

Pipeline Original and Desana Verify were the two best performing cleaners with respect to biofilm reduction on each line material and reduction in loading of planktonic microorganisms in dispensed beer after cleaning. Both cleaners performed best on the Barriermaster Flavourlock lines and this line material type had lower biofilm levels for all the cleaners and also reduced loading of beer ex dispense.

With respect to the recommendation that Molson Coors would be able to extend line cleaning frequency to four weeks if using a dispense system at extra cold temperatures combined with chilled fonts and the Barriermaster Flavourlock line, it is recommend that cleaning of the system is performed with either Pipeline Original or Desana Verify.

7 THE MICROBIOME OF A BEER DISPENSE SYSTEM

7.1 Introduction

Draught beer and dispense systems can be compromised by the growth and metabolic activity of contaminating microorganisms. Bacteria and yeasts have been identified as beer spoilage microorganisms such as *Lactobacillus*, *Acetobacter* and *Brettanomyces*. Dispense systems are exposed to environmental contaminants in the bar at the tap/nozzle and in the cellar at the keg coupler/spear. Cask beer can also be contaminated from airborne microorganisms when on sale as air is pulled through into the cask during dispense.

Microorganisms present in draught beer can cause numerous quality defects affecting both appearance and taste. Whether it be haze formation due to excessive growth or the development of off-flavours and aromas caused by their metabolic activity. Between periods of line cleaning, beer dispense systems can become colonized by both yeast and bacteria that can form biofilm(s).

Biofilm formation begins with an initial surface interaction causing cells to transition from planktonic to sessile (surface attached), these cells can then support each other. Fermentative organisms will utilize available sugars to produce organic acids which are then utilised by the metabolism of other species in the biofilm. As

4507 the community grows the formation of protective
4508 exopolysaccharides covers the biofilm with a slime like substance
4509 making it more resilient to cleaning and antimicrobial substances.
4510 Once mature, biofilms can shed flakes of cells to colonise surfaces
4511 further along the line (Davey and O'toole, 2000; Quain and
4512 Storgards, 2009).

4513 Beer related biofilms have predominantly been isolated and
4514 recovered from within the brewery, particularly in packaging halls.
4515 Poor hygiene in these areas leads to risks of final product
4516 contamination especially where aseptic filling is practiced as there
4517 is no further microbial stabilisation (e.g. pasteurisation). Bottle fillers
4518 and conveyors for example, have been found to become
4519 contaminated with biofilm in just a few hours (Storgårds *et al.*, 2006;
4520 Storgårds and Priha, 2009; Priha *et al.*, 2015).

4521 Very little data has been presented so far on the complex
4522 community of microorganisms found within beer dispense systems.
4523 Published work on microbial attachment to beer line and stainless
4524 steel has been performed with laboratory cultures (Percival *et al.*,
4525 1998; Godfray, 2005; Walker *et al.*, 2007). Work presented here is
4526 very much a first look at the inherent variety that can be present in
4527 the 'real world' dispense systems.

4528 Draught beer (Carling) from the on-trade was incubated and fed
4529 with fresh media (fresh Carling ex can) to encourage growth of the

indigenous microorganisms. This was then diluted and used to inoculate a dispense system (see 2.5.2.2). After a series of trials (Chapter 6), the system was sampled for its microbial composition, beer was taken from the tap and directly from the kegs supplying the system. The beer lines were cut, and sections removed for biofilm recovery and identification as per method 2.5.4.1, after vortexing the swab 100 µL of the Amies solution was pipetted onto agar plates to culture the present microorganisms. Samples were taken from five lines; Control (6-8°C, MDP), S1 (6-8°C, Barriermaster Flavourlock), S2 (6-8°C Brewmaster 2), E1 (1-4°C, Barriermaster Flavourlock) and E2 (1-4°C, Brewmaster 2).

Samples were cultured on three different media; Wallerstein nutrient agar (WLN), Raka Ray (RR) and Plate count (PCA) they were incubated at 30 °C both aerobically and anaerobically. WLN and PCA plates were incubated for 72 hours whilst Raka Ray was incubated up to a maximum of seven days as some lactic acid bacteria can be slow growing.

163 single colonies were then picked and streaked to pure culture, ready for DNA extraction (see 2.4.1). PCR was used with either of two sets of primers, ITS 1 & 4 for yeast and 63F/1387R for bacteria, the amplicons produced were then sent for sequencing. A total of 114 were successfully sequenced and identified with a minimum 95% confidence.

7.2 Results & Discussion

7.2.1 Culture of microorganisms from keg, line and glass

Initial variation between the microflora present from the three sample points could be seen from the growth on media plates (Figure 7.1, 7.2 and 7.3).

The biofilm plates incubated aerobically (Figure 7.1) showed the most variation. The control line constructed of MDP and lines S2 and E2 both constructed of Brewmaster 2 beer line showed good yeast growth coupled with bacterial colonies. Lines S1 and E1 constructed of Barriermaster Flavourlock - a line designed to reduce the ingress of oxygen and maintain levels of dissolved CO_2 - contain lower levels of growth and less diverse colony morphologies. An obvious difference however is the presence of a reddish/pink colony type present on S1 only.

The anaerobic plates from the biofilm (Figure 7.3) show similar colony morphologies across the three different line material types but after this anaerobic incubation, many more colonies can be seen on the E1 plate suggesting a greater presence of anaerobes in the biofilm. This fits well with the expectation that the gaseous atmosphere within this line would be more anaerobic.

Growth from the beer ex dispense was similar across lines S1, S2, E1 & E2 with the control line showing increased numbers of yeast colonies. The control line at the time of sampling had the greatest

biofilm level and the worst quality beer ex dispense. With such a mature biofilm present it is likely that there were more incidents of pioneer cells be it yeast, or bacteria being shed during the turbulent flow of beer dispense and therefore a higher incidence of planktonic yeast in the beer in glass.

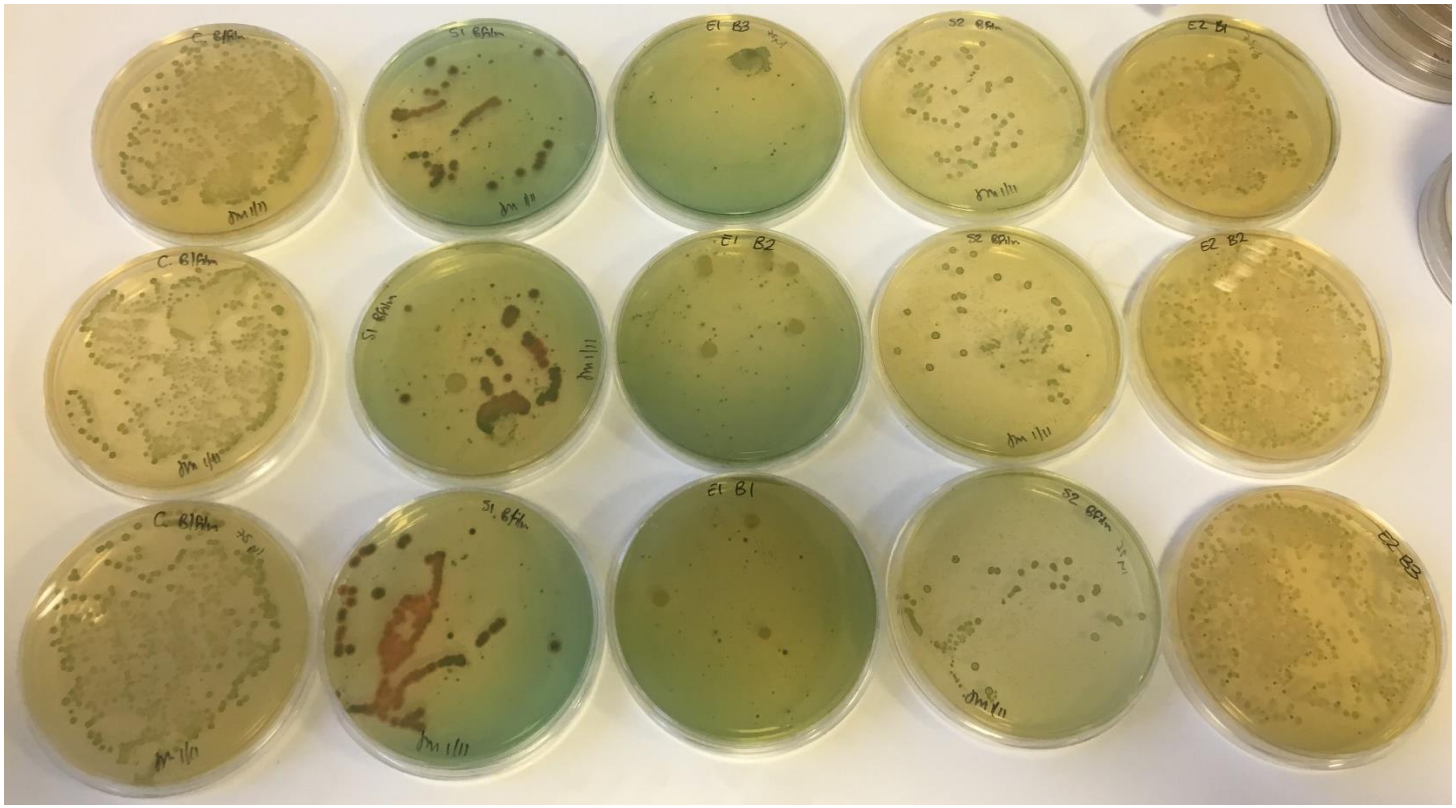


Figure 7.1: Growth of microorganisms present on biofilm swabs from a beer dispense system. Wallerstein Nutrient agar incubated for 72 hours at 30°C. Columns read from left to right as C, S1, E1, S2, E2.

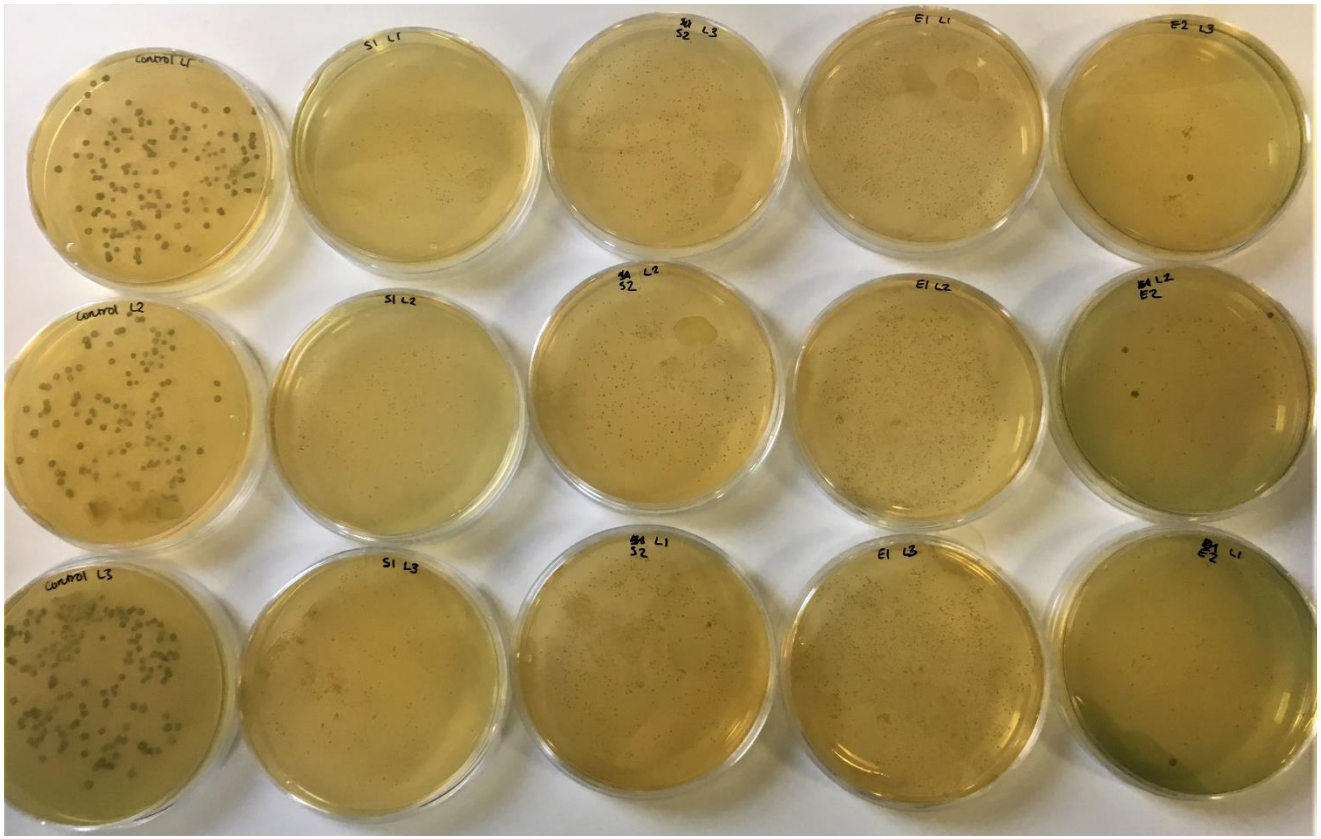


Figure 7.2: Growth of microorganisms present in dispensed beer. Wallerstein Nutrient agar plates incubated for 72 hours at 30°C. Columns read from left to right as C, S1, S2, E1, E2.

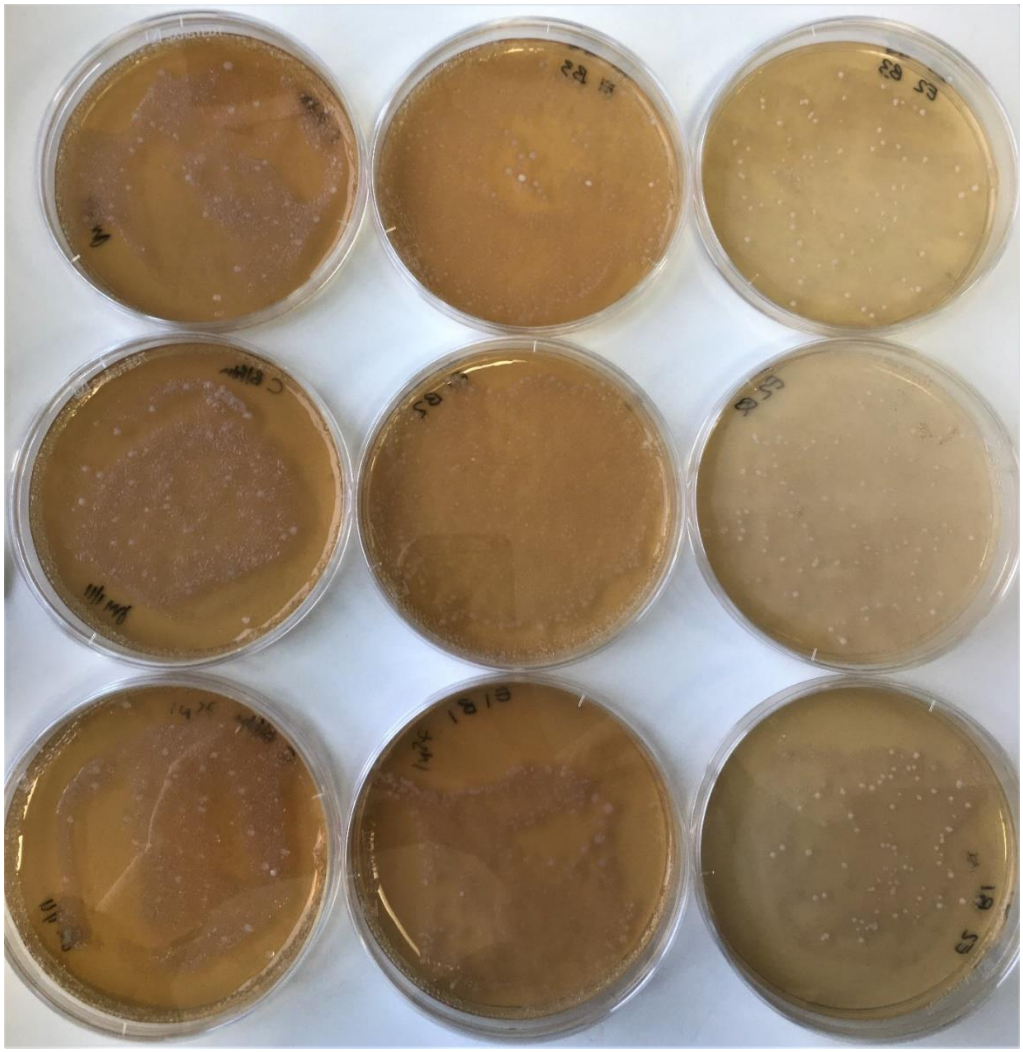


Figure 7.3: Raka Ray plates, showing microorganisms present in the biofilm, lines C, E1 and E2 respectively, incubated for seven days at 30°C.

7.2.2 ITS & 16S PCR

The DNA was extracted from 163 pure cultures, the yeasts were extracted using the method outlined by Lõoke *et al.,* (2017) whilst bacteria were extracted using phenol-chloroform (Legras and Karst, 2003). The extracted DNA was then subject to PCR to amplify

the ITS and 16S regions of yeast and bacteria respectively. These regions are highly conserved and can be used to identify at the species level (Pham *et al.*, 2011; Siegrist *et al.*, 2015).

Amplicons were then observed on agarose gels to confirm successful amplification, bacteria amplicons were in the region of 1500 kb size (Figure 7.4), whereas yeast dependent upon strain were in the region of 400 to 900 kb (Figure 7.5). All gels contained a 1kb ladder for comparison.

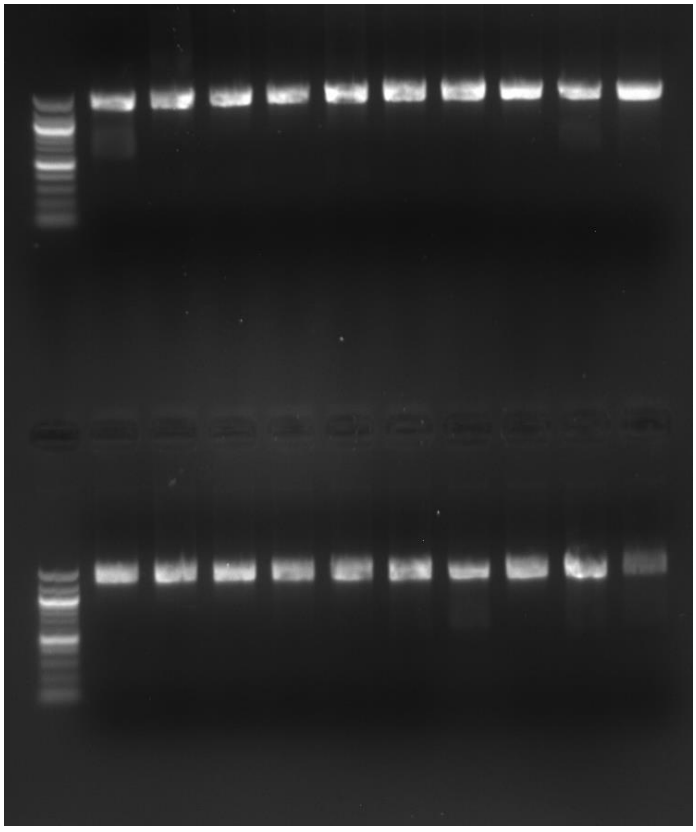


Figure 7.4: Bacterial PCR products observed under UV light on an agarose gel.

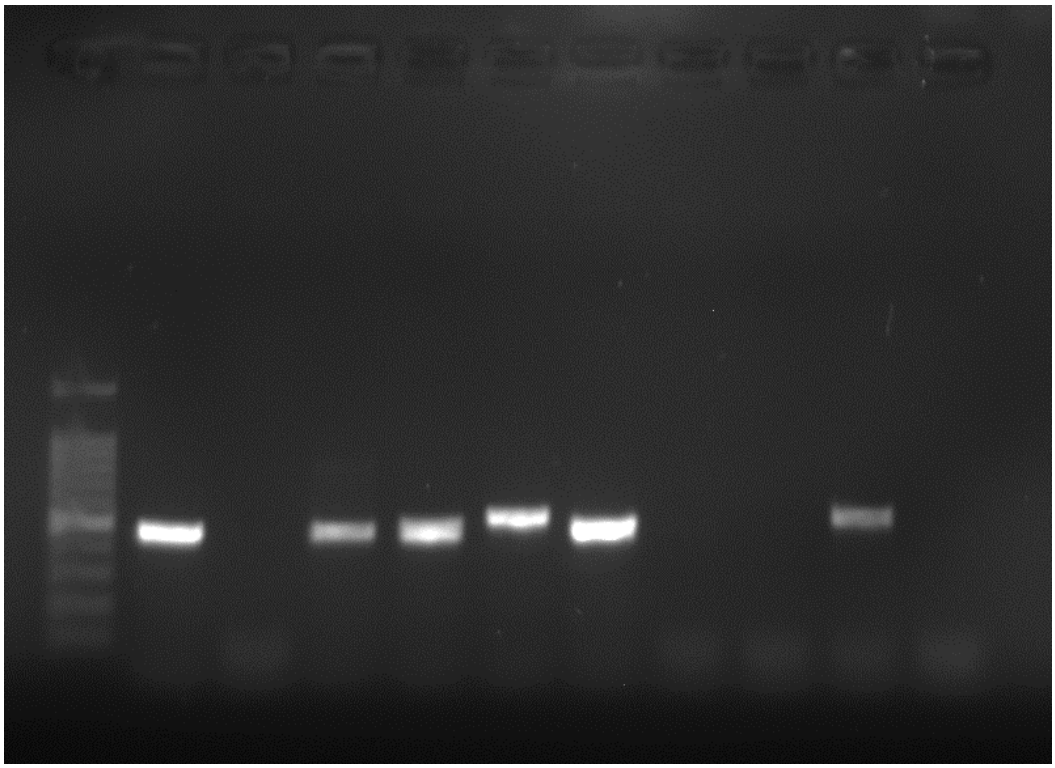


Figure 7.5: Yeast PCR products observed on an agarose gel.

Following PCR, 126 products were successfully replicated, these were purified and diluted to a DNA concentration of 20 ng/µL and sent for sequencing by an external service provider.

7.2.3 Species identified in keg, line & glass

Following sequencing, 114 samples were identified with a 95%+ certainty. Of these 40 were yeasts and 74 bacteria.

Table 7.1 Microorganisms identified from each sample point.

Sample point	Keg	Line (Biofilm)	Glass
Yeast	11	21	5
Bacteria	15	24	38
Total	26	45	43

7.2.3.1 Species identified in keg

Three yeast genera were identified in the keg samples. Two species of *Pichia*; *P. membranifaciens* and *P. manshurica*, *Brettanomyces bruxellensis* and *Yarrowia lipolytica*. *Pichia membranifaciens* is the type species of the genus and associated with forming biofilms, its sibling *P. manshurica* is closely related and there has been debate about whether they are the same species (Naumov and Naumova, 2009). *Pichia* has been found in dispense systems previously (Harper, Hough and Young, 1980; Casson, 1982, 1985) and is a producer of flavour compounds such as ethyl acetate and phenyl ethanol giving a fruity or solvent-like aroma when produced in excess (Capece *et al.*, 2018). *Brettanomyces bruxellensis* is commonly associated with beer spoilage and is associated with a phenolic off flavour production as it generates 4-vinylguaiacol and 4 ethylphenol alongside copious amounts of acetate (Gilliland, 1961; Hall, 1971; Bokulich and Bamforth, 2013). Finally, *Yarrowia lipolytica* has never been associated with beer previously, despite it being an aerobic, dimorphic yeast capable of growing in many environments. It is normally associated with fats and oils and produces lipase as part of its metabolism (Gonçalves *et al.*, 2014). This would suggest that the presence of this microorganism is as an external contaminant picked up in sample collection or culture.

Bacterial contaminants found in keg were *Acetobacter indonesiensis, Lactobacillus brevis, L. paracollinoides, Bacillus cereus* and *B. thuringiensis*. *Acetobacter* species are ubiquitous in the environment and are prolific beer spoilers, the same can also be said of *Lactobacillus* species. They have fermentative metabolism and both genera produce acetic and lactic acids whilst *Lactobacillus* can also produce diacetyl.

Bacillus cereus and *B. thuringiensis* are both spore forming bacteria and members of the *B. cereus sensu lato* group, they carry the gene identified in *Lactobacillus* species as conferring hop resistance (Haakensen and Ziola, 2008). They have previously been isolated from beer and their likely route is through contamination of malt crops, they are often used as pesticides for crops and in the forestry sector (Kuroki *et al.*, 2009) their spores can survive pasteurisation during beer production and they may then sporulate later. In beer dispense though they are more likely to be environmental contaminants. Due to the pH of beer, they are. unable to grow in beer. It is a concern though that *B. cereus* (a recognised human pathogen) has been identified and suggests that inadequate beer hygiene may be a potential health concern (Haakensen and Ziola, 2008; Maifreni *et al.*, 2015; Munford *et al.*, 2017).

7.2.3.2 Species identified from the dispense line biofilm

The biofilm isolates contained the greatest variation in microflora, with some organisms not recognised as beer spoilers. Again *B. thuringiens*is was present, it can form biofilms along with its fellow pathogenic strains *B. cereus and B. anthracis*. One study by (Auger *et al.*, 2009) found that 42% of strains were capable biofilm formers but all of which were non-pathogenic.

Paenbacillus humicus was also identified, like *Bacillus* species, it has been associated with beer, although not directly with beer spoilage. *Paenbacillus humicus* was found to possess the *HorA* gene providing hop resistance and it was found to cause turbidity in beer after 20 days of growth (Haakensen and Ziola, 2008). It has a very diverse metabolism allowing it to occur and survive in beer (Munford *et al.*, 2017). It is a good candidate to be found in biofilms as it can produce enzymes (mutans) that breakdown glucans, which would enable other members of the biofilm to use these products for their own metabolism (Grady *et al.*, 2016; Munford *et al.*, 2017). *P. humicus* was only identified in the extra cold beer lines and, because colonies were recovered in abundance, this suggests it could grow better than competing microbes at the colder temperature. Indeed, this *Paenbacillus* species has been found to grow well below 6°C and are regular contaminants of dairy processing plants and milk (Grady *et al.*, 2016).

Rhodotorula mucilaginosa, was found in three of the five lines, its characteristic reddish pink colonies are visible on the WLN plates in Figure 7.1, it is a primary biofilm coloniser and has been recovered from brewery filling hall biofilms. It is associated with damp locations and is often seen in washing machines, water pipes and showers (Gattlen *et al.*, 2011).

Saccharomyces cerevisiae and *S. uvarum* were identified in the biofilm, brewing strains are not readily associated with forming biofilms (Riedl *et al.*, 2019) however, they can be associated with the exopolysaccharides (EPS) generated by primary colonisers. EPS is a slime-like substance and affords the biofilm protection and will capture planktonic cells that are present in the line. *Saccharomyces* is unable to attach to inert surfaces however under stress, starved cells express the *Flo11* gene much more strongly and this enables surface attachment. They can also attach when biopolymers are present (Wood *et al*., 1992; Douglas *et al.*, 2007; Riedl *et al.*, 2019).

50% of the microorganisms identified belonged to either the Acetic acid bacteria (AAB) or the Latic acid bacteria (LAB). Members of the AAB identified were *A. malorum, A. fabarum and G. oxydans*. AAB are often cited as the primary colonisers in brewery biofilm, they are Gram negative aerobic organisms and like many Gram negative genera are capable of EPS production aiding in biofilm

development (Back, 2003; Storgårds *et al.*, 2006; Quain and Storgards, 2009).

LAB namely, *L. brevis and L. paracasei* were common to the biofilm in all lines, despite not being reported as primary colonisers they are regularly found adhered to beer dispense lines (Harper *et al*., 1980; Casson, 1985). They are the causative organism of most of the beer spoilage events that have been reported (Sakamoto and Konings, 2003; Suzuki, 2015). They produce excessive amounts of lactic and acetic acids dependent on whether they are hetero or homofermentative strains.

The biofilm has been found to contain several recognised beer spoilers and some environmental contaminants. The real number of species present in the biofilm will likely be far greater than presented here. A survey of biofilm performed using both culture-dependent and independent methods highlighted that the independent methods found a much higher variety of microorganisms (Timke *et al.*, 2008; Carraro *et al.*, 2011). This is likely to be due to the nutritionally fastidious nature of some species and the issues surrounding viable but non-culturability (Suzuki, Iijima, Asano, *et al.*, 2006).

7.2.3.3 Species identified from beer ex dispense

The most prolific contaminant in beer ex dispense during this study was *Gluconobacter oxydans* followed by *L. brevis*, belonging to

either the acetic acid bacteria (AAB) or lactic acid bacteria (LAB) (see 1.5.2.1 & 1.5.1.1). Out of 42 isolated microorganisms only 4 were yeasts: *S. cerevisiae, B anomalus* (x2) and *B. bruxellensis*. Again, these organisms are commonly associated with beer and beer spoilage and would be expected to be part of the microflora of most beers post dispense.

7.3 Conclusion

Work presented here is the first investigation of the culture-based microbiome of a beer dispense system, comparing the flora found in the keg, beer line and final product served to the consumer. As expected, the commonly associated beer spoilage organisms such as members of the AAB and LAB along with the wild yeast genus *Brettanomyces* were all present in all parts of the dispense system.

The greatest variation in flora was found to be in the biofilm recovered from beer dispense lines. Within this the spore forming bacteria *Bacillus cereus* and *B. thurigiensis* as well as *Paenbacillus* sp. were identified. These organisms are not recognised beer spoilage organisms but have been linked to human pathogenicity. It is likely that the inherent pH and the other anti-microbial conditions in beer mean these bacteria cannot grow in beer, but their presence is a concern from a hygiene perspective.

It is suggested that the work presented here is an under estimation of the microbial community found within the beer dispense system. Colonies were picked on abundance to highlight the most common organisms present using culture dependent techniques. Unpublished work using culture independent techniques, performed alongside D. Quain and fellow PhD student A. Jevons, has highlighted that the microbial community is much more complex than is revealed here.

8 CONCLUSIONS AND FUTURE WORK

8.1 Conclusions

There are many pressures on the beer retail sector (on-trade), a myriad of reasons be it political, social, economic or technical exist. However, beer quality and the assurance of it is an ever-present issue. Anecdotally, it is accepted that the quality of draught beer at the point of sale varies and it is likely that consumers will have had a negative experience on one or more occasions. Issues with low rate of sale, cleanliness/hygiene, maintenance of the cellar temperature and associated dispense equipment can all be root causes of poor beer quality and consequent poorly performing retail accounts.

The primary aim of this three-year project was to produce for the first time a quantitative assessment of the state of draught beer hygiene in the on-trade, raise awareness and improve best practices through communication by publication of papers, magazine articles and conference attendance together with this thesis.

Chapter 3 presents a simple yet effective method for measuring the hygienic status of draught beer. The method provides a direct measurement of the microbial loading present at the point of sale, which, upon incubation, grow increasing the turbidity as measured at OD_{660nm}.

The method supports those microorganisms that can grow and spoil beer as they are grown in the media (beer ex dispense) from which they are sourced. It is suggested that this is a useful reference method to assess the hygiene of the beer (and the dispense system), rather than the traditional industry standard spread plating techniques. These use a range of specific culture media which are 'selective' and accordingly do not support the growth of all beer spoilage microorganisms. Further, the use of 'plates' can be further compromised by the presence of viable but non-culturable microorganisms and nutritionally fastidious genera such as *Lactobacillus* (Suzuki, Iijima, Asano, *et al.*, 2006; Asano *et al.*, 2007).

After validation, the method, was applied in a wide-ranging survey of draught beer from on-trade accounts in the East Midlands. Beer quality was assessed in an array of locations, account types and price brackets. Novel insights were gathered from the market place including ; the wide variation in draught beer quality, the variation in susceptibility to spoilage of differing beer styles, the inverse relationship between price and quality, the negative impact of having too wider range of beers available ('over-fonting') and that the industry standard award for cask beer quality ("Cask Marque') had no beneficial effect on the quality of keg beer. This might be expected as in such accounts it is assumed

that their better practices in cellar management and cleanliness would extend to keg beer.

Three accounts were further investigated in Chapter 5, whereby routine sampling of beer quality across the bar, pre and post line cleaning occurred providing a base level of quality for each account. Best practice measures were then introduced to each account and the impact measured by repeat sampling of the accounts again, pre and post clean over several weeks.

In addition to line cleaning, the best practice measures were focussed on the two ends of the dispense system, keg connection interface consisting of the keg spear and keg coupler, and the tap on the bar which, in the majority of cases consists of a font body with spout and removable nozzle.

The two ends of the system are likely entry points for environmental contaminants. Product spillages and stagnant water can contribute many microorganisms and airborne contaminants in the cellar can also contaminate cask beer as air is drawn into the cask as part of the dispense process.

In terms of the bar, airborne microorganisms can find their way to the open end of the tap. Taps are rarely covered or plugged in the UK. There is also regular contact with bar staff, who handle nozzles and cask beer sparklers. This is a concern for cross contamination as they may also be serving food (and recovering

plates) to customers. Some consumers request that the nozzle/sparkler be removed prior to dispense which again exposes the nozzle to direct contact with staff, who may have handled money, food and other items. Further and alarmingly, lapses in personal hygiene could potentially introduce contaminants to draught beer.

The rate of sale and its effect on beer quality and the consequent speed of recontamination of beer dispense lines was assessed. It is accepted that in a brewery with appropriate quality assurance regimes that kegs on leaving the brewery will be 'commercially sterile', containing <1 cfu/L (Boulton and Quain, 2001). Work presented here found that upon connection to a dispense system, microbial contamination in the keg occurred. It is suggested that as the tap shuts and beer flow stops, the resultant wave generated in the beer line, causes the ingress of a small volume of beer back into the keg through the 'non-return' valve in the keg coupler device. Further support for this proposed method of keg contamination was gained by measuring the level of contamination present after an increased number of open/closing events. The level of potential growth was found to increase in line with an increasing number of these events.

In an ideal scenario it would be better for accounts to only attach un-broached new kegs post line cleaning to extend the time to

reach re-contamination. Using a new keg would remove the reservoir of microorganisms that is able to accumulate in the keg which has been on the dispense system for a number of days. However, both practically and commercially such practice is completely unviable.

Best practice goals defined by this body of work are:

- Clean dispense lines every seven days, ensuring the correct dilution of line cleaner is used.
- Sterilise dispense tap nozzles at the end of each day of trading. This should be performed using a commercially available chlorine based sanitising tablet, dissolved in cold water. This achieves 'commercially sterile' nozzles compared to the impractical line cleaner and carbonated 'soda' water which exacerbates the growth of microorganisms.
- Spray the keg coupler and keg spear with a commercial food-grade sanitiser prior to each connection between line and keg.
- Keg turnover should be within a maximum of seven days. Whilst a cask should be emptied in no longer than three days.

- Where possible, simplify the dispense system reducing the number of split lines and thus reducing the number of 'dead legs' in the system.
- Where many taps are used for a brand, ensure that each line is used to keep beer moving or consider a reduction in the number of beer lines connected. Alternatively, gas lock lines during the quiet period of the trading week (Sunday to Wednesday).
- Detach any taps that are located on a rarely used bar such as a function room bar. These lines should be gas blocked and not left in water (which becomes contaminated!)

These recommendations were applied to the accounts involved in the study and, as was expected, the introduction of best practice triggered an improvement in beer quality across the bar. The accounts in some cases achieved higher quality scores for the beer pre- cleaning than was previously achieved after cleaning.

The next phase of the project focussed more on potential innovation within the dispense system. Innovations around line material, dispense temperature and cooling all the way to the tap were assessed. Using the method validated in Chapter 3, biofilm quantification and gas chromatographic analysis of the volatile profile(s). Beer and beer line segments from the novel dispense

system(s) were assessed and the hygienic improvement comparable to a traditional U.K. dispense system measured.

A combination of components enabled line cleaning frequency to be extended from seven days to 28 days with confidence that there would be no adverse effect on the quality of the dispensed beer compared with the beer quality at seven days. Use of the Barriermaster Flavourlock line with reduced gas permeability coupled with cooling to the back of the tap and an extra cold dispense temperature of 1 – 4 °C provided a dispense system with reduced biofilm formation and off flavour production, which achieved similar or better results to those seen with the traditional dispense system at seven days after cleaning. Therefore, it was recommended that these conditions are used in future dispense system installations where an extended cleaning frequency is desired.

Four commercial line cleaning solutions were investigated in a comparative study. Their efficacy was assessed by measurement of the microbial load in glass and quantification of biofilm pre and post cleaning. Two line cleaning solutions proved to effectively reduce biofilm level and reduce loading of microorganisms in dispensed beer. Both are caustic based detergents with an indicator to detect the presence of organic matter. Accordingly,

Pipeline Original and Desana Verify were recommended line cleaning solutions based on findings in Chapter 6.

The final investigation looked at the constituent members of the microflora found within the dispense system used in Chapter 6. Beer in glass, biofilm and beer sampled directly from the keg were cultured on media and microorganisms identified.

The common beer spoilage bacteria: *Acetobacter, Gluconobacter* and *Lactobacillus* were found across the dispense system along with the wild yeast *Brettanomyces*. The greatest complexity of microflora was in the biofilm whereby environmental spore-forming *Bacillus* were found alongside the yeast *Rhodotorula mucilaginosa* which is often associated with moist environments such as water pipes and washing machines. The study used a culture dependent approach and therefore is likely to be an underestimation of the complexity. To progress this moving forward it is suggested to use culture-independent techniques, thus reducing the impact of the VBNC state and inability to detect low level contaminants.

8.2 The future

The work presented here has covered the breadth of beer dispense and associated practices. Further investigations that would expand and enhance the understanding gained in this project would be:

8.2.1 Beer composition and its selectivity for a different spoilage microflora

An obvious theme running through this thesis is that different beer brands spoil at different rates. Evidence from the on-trade investigation shows variance not only between beer styles but, also within beer styles.

Finished beer will contain varying levels of compounds that either aid or inhibit the growth of diverse microorganisms. Things to consider include the residual sugars (including dextrins), that are low in lager style beers but higher in ales with more body. Further considerations include the levels (and mix) of organic acids which can act as nutrient sources and melanoidins which are high in darker beer styles such as stouts and porters.

Currently unpublished work suggests that some beer brands are microbially more robust than others. An understanding of what the key factor(s) are in its composition that drive this robustness, whether it be the raw materials or a specific process in the production, could lead to designing draught beers that are more robust to spoilage.

8.2.2 Is the microflora driven by the brand or the account?

A build on the above experiment, would be to then understand the microflora found within a specific brand and how conserved it is across various accounts. Do the same species repeatedly

appear in a brand across many accounts or is it the accounts inherent microflora that pre-dominates. It is suggested that each brand would self-select its spoilage flora from the available microflora within the account as Baas Becking (1934) stated 'Everything is everywhere but, the environment selects.'

8.2.3 Sensory analysis of draught beer – consumer awareness

The consumer perception of off flavours and aromas is little understood, particularly as consumers become more educated about beer.

For example, using an untrained panel to gain consumer insights, their ability to identify obvious signs of spoilage and the feelings and responses this develops whether they blame the brand of beer or the outlet itself for a poor experience. The industry - accounts and brewers - could then understand how important it is for brand reputation and outlet performance to provide consistently good beer quality.

This insight coupled with an understanding of the levels of microorganisms needed to generate obvious off flavours and aromas would complete the picture of draught beer spoilage.

8.2.4 Innovation in dispense cleaning practices

8.2.4.1 Increasing turbulence during cleaning

Line cleaning equipment that circulates the liquid during cleaning with increased turbulence are rarely used in the UK. However, outsourcing line cleaning to independent contractors has seen an increase in circulating systems.

In brewery CIP systems (and domestic dishwashers!) the effectiveness of cleaning is derived by the temperature, time, the chemical treatment used and the mechanical action applied (Davies *et al.*, 2015). With line cleaning, cold water is used as hot water is typically unavailable in most cellars. Interestingly, Ringwood in 1924 suggested the use of hot water for line cleaning. Although manufacturers of line cleaning solutions suggest concentrations and contact times for their products which, if followed, they should work effectively. The remaining part of the jigsaw is how much more effective line cleaning could be if turbulence/mechanical action was generated in the line as well.

8.2.4.2 Enzyme pre-digestion of biofilm

Enzymic pre-digestion of biofilm proved to reduce the loading in the line after cleaning, when compared with lines not pre-treated in a study by Walker *et al.*, (2007). The enzyme-based cleaner used in Chapter 6 however, performed poorly in comparison with the other line cleaners and it suggests work is needed to define the

best use of enzymes in cleaning. The biggest challenge of such an approach is the assumption that biofilms are the same which – if driven by beer composition – is unlikely.

8.2.4.3 Effectiveness of electromagnetic/ultrasonic waves in preventing biofilm formation

Currently unpublished work in one of the three accounts used in Chapter 5, shows the introduction of an electromagnetic based system applied to the keg lines between keg and FOB were able to extend the cleaning frequency with no adverse effect on quality. It is suggested that the electromagnetic waves cause turbulence in the beer line reducing the ability for microorganisms to settle and form biofilms on the beer lines. As the effect is likely to be local this approach may impact on the growth of microorganisms and the recontamination of kegs with dispense. Greater understanding of how this technology works could lead to further innovation built-in to novel dispense systems.

8.3 Final remarks

Assurance of draught beer quality and effective cleaning of dispense systems has received very little attention since the work Hough and colleagues from the British School of Malting and Brewing at the University of Birmingham in the 1980s and by Erna Storgards from VTT in Finland in the late 90s. This project should be considered as a starting point to reignite the discussion around an often-neglected part of the brewing world. It lays the foundation

for further focussed research in this area.

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APPENDIX I

Table S1 Supplementary information.

Method development	Brands	Licensed premises
Universals	ST1 PL7	B7
Reproducibility	SL 3, 4, 6 PL 1, 5 SKA 1, 5	B 1, 2, 6, 8, 9
Static v mixed incubation Incubation time	SL 1, 3, 4, 6, 8 SKA 1, 2, 3, 5 SL 1, 3, 5 SKA 1, 5	16 including D 1-4, 6-10, 12-16, 18, 19 10 including D 4, 5, 6, 10, 12-16, 19
Overnight storage	SL 6, 7 PL 3 SC 1, 4, 7, 8 ST 2 C 3, 4	L 7
Cask and turbid beers	SC 1, 3, 4, 5, 7, 8 WH 1, 2 CR 1, 2	Cask, V 14, L 7; Wheat beer, D 12 Unfiltered beer B 10
Microbiology and forcing	SL 3, 4, 5, 6 SKA 1, 3, 5 PL 1, 2, 3, 4, 5 SC 7 C 3, 4	B 2, 5-8; D 4, 6, 7, 10-16, 18, 19; L 7 and V 4, 6-9

Where brands are segmented as Lager < 4.1% abv (SL), Lager > 4.1% abv (PL), Ale < 4.1% abv (SKA), Ale >4.1% abv (PKA), Cask Ale (SC), Stout (ST), Wheat beers (WH), Craft/microbrewery beers (CR) and Cider (C).

Samples were taken from on-trade licensed premises in Burton-on-Trent (B), Derby (D), Loughborough (L), Market Harborough (MH) and villages (V) between Burton and Derby.

APPENDIX II – Incubation time required for forcing test

Foreword:

Three brands of nationally available beer and two of cider were inoculated with micro-organisms frequently found in beer. A microtiter plate reader was used to mimic the forcing test conditions and growth curves plotted for each organism in each brand.

Findings:

- Confirmed that 96 hours was sufficient incubation time for growth of all organisms as per the forcing test.
- Bacteria were unable to grow in cider; this was expected due to inherent cider pH of ≤ 3.8
- Increased sugars in Strongbow Dark Fruit aided in growth of both species of Saccharomyces yeasts.
- Lactobacillus grew similarly in all three beers, regardless of beer style.

TECAN data and growth curves:

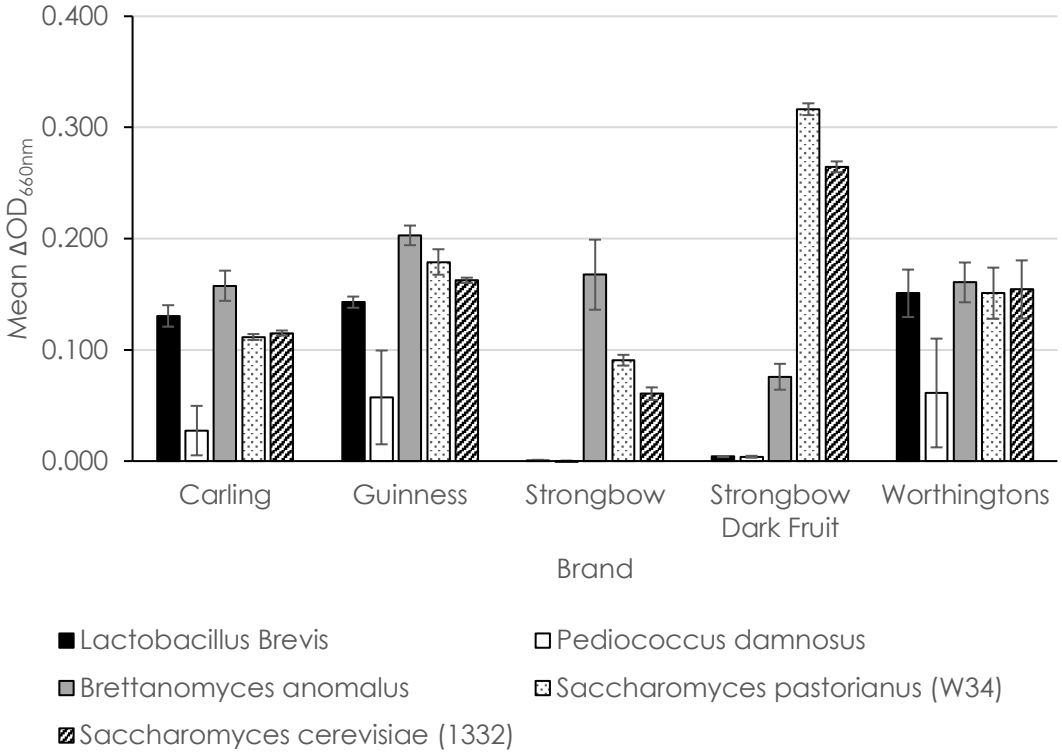
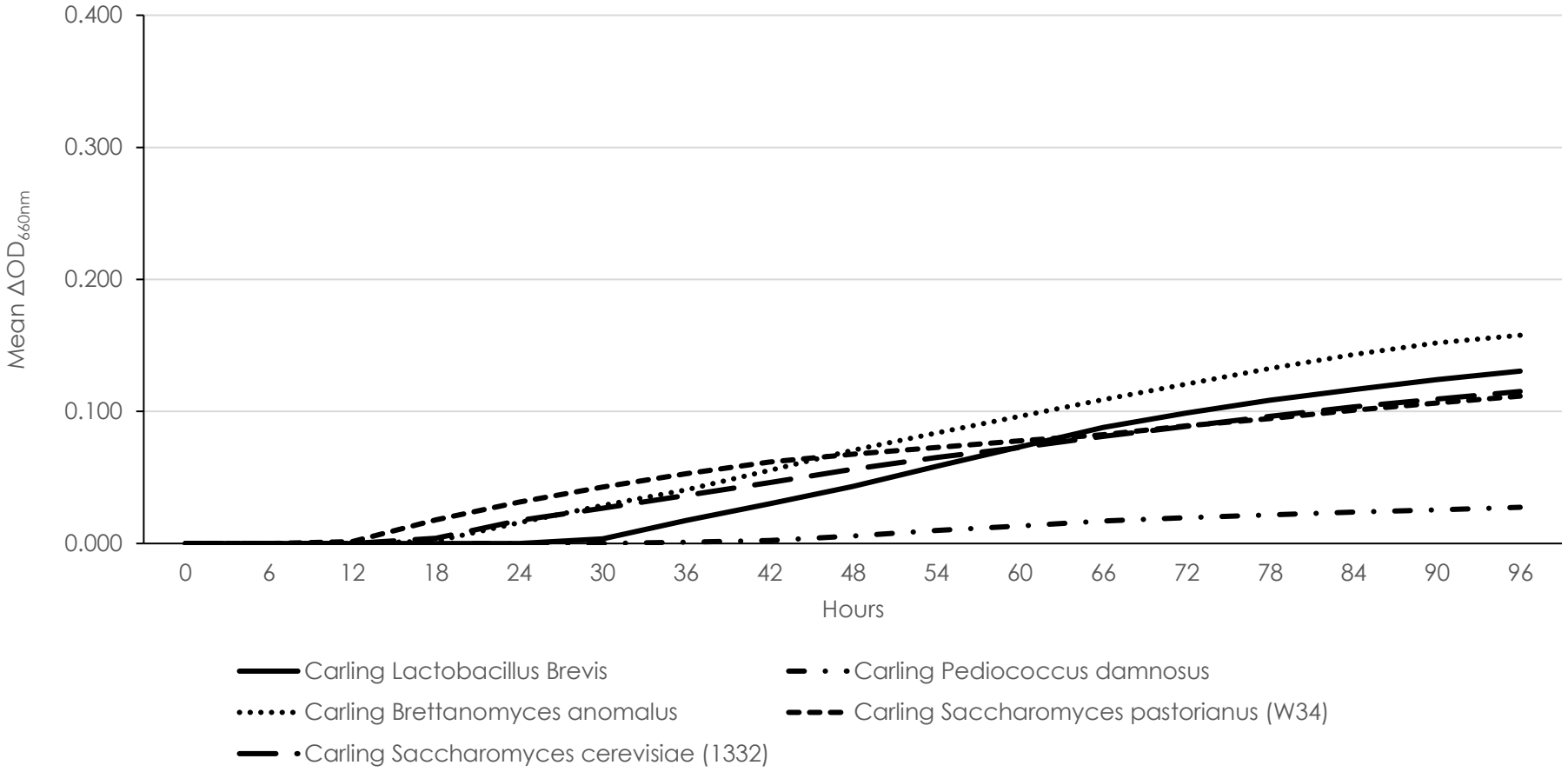
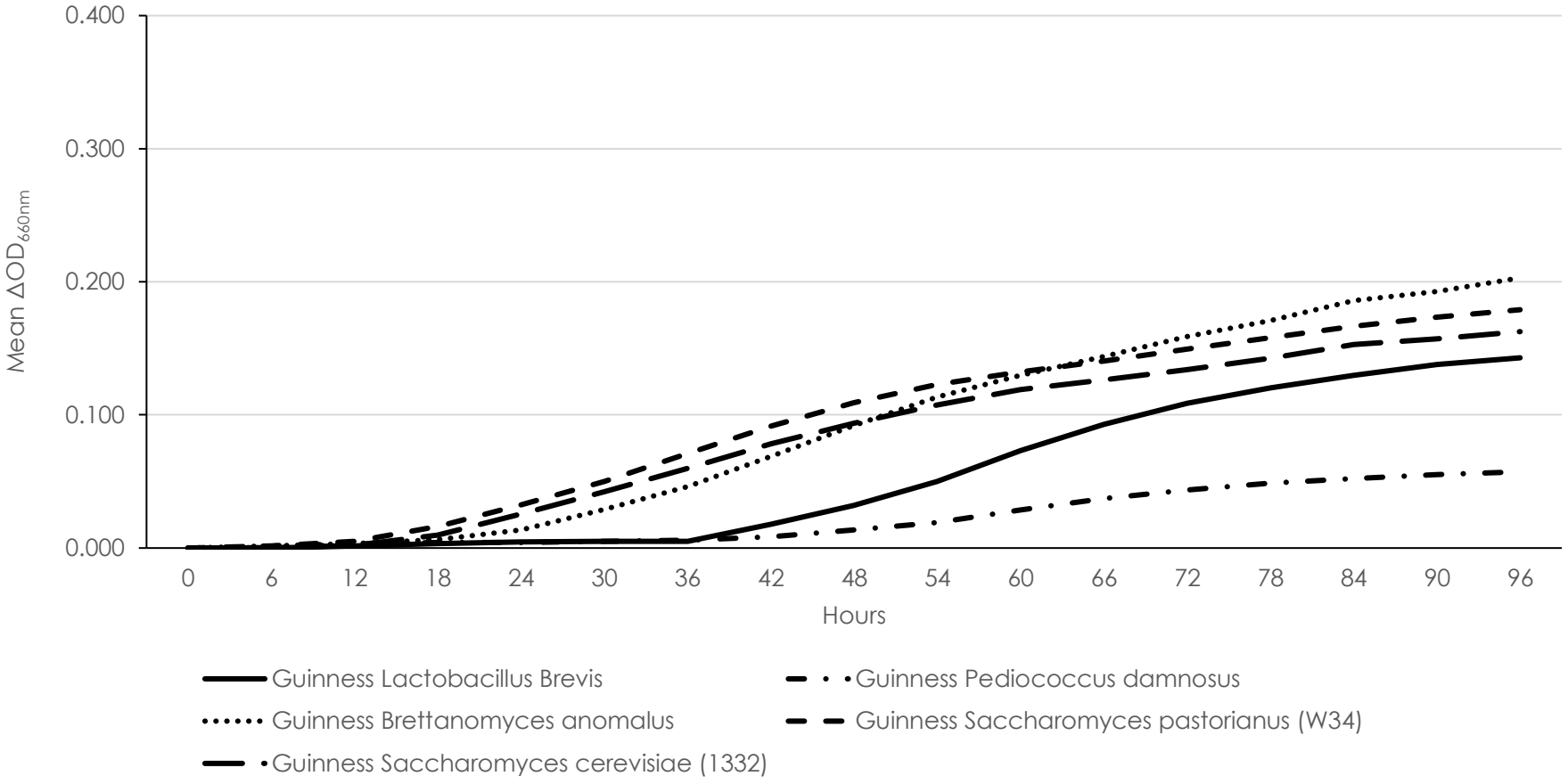


Figure Ai.1: Mean ΔOD_{660nm} observed when five different micro-organisms were independently inoculated in to five different brands of beer and cider.



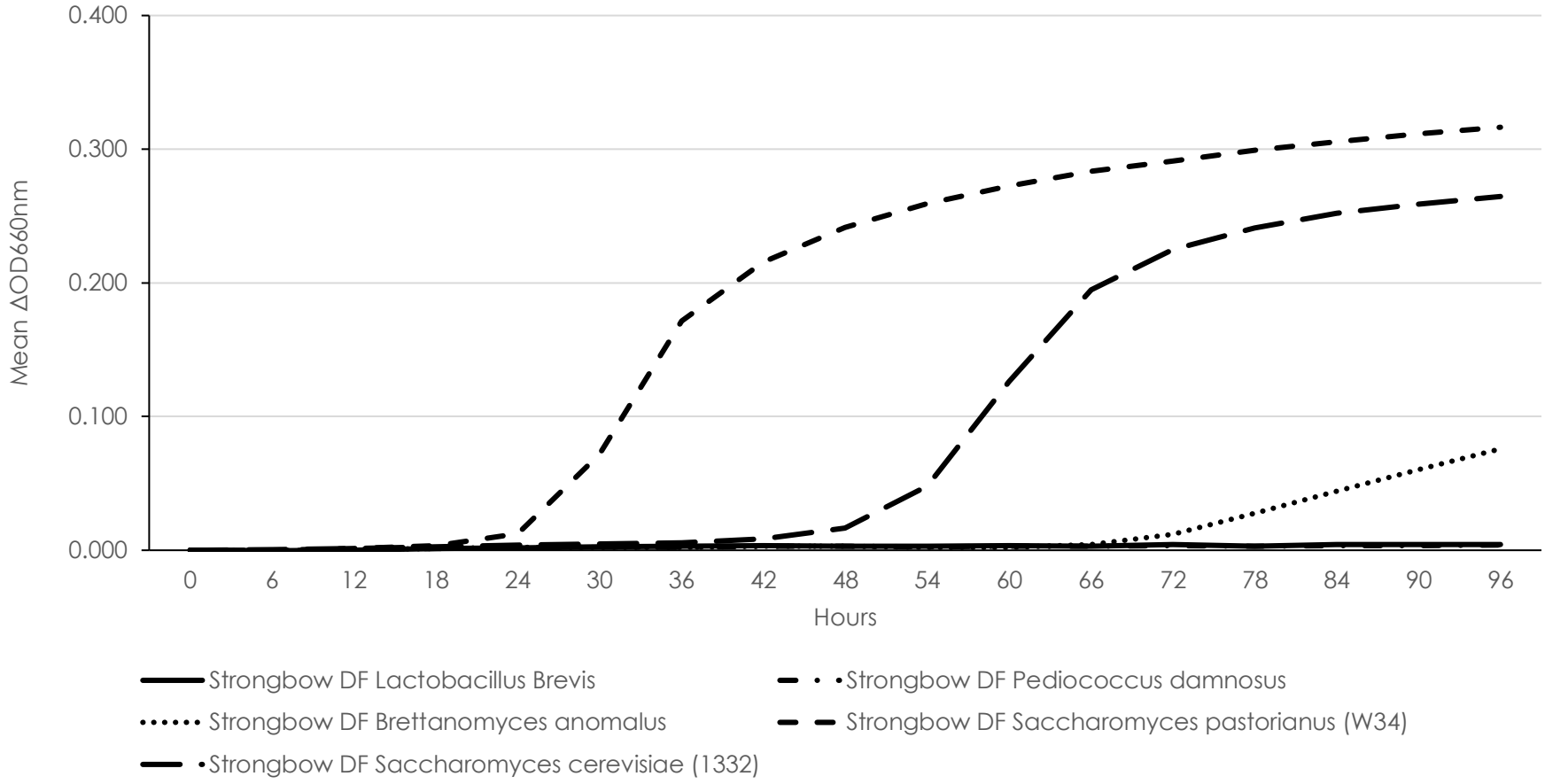
5828

5829 Growth curves of five different micro-organisms in Carling beer, at 37°C for 96 hours.



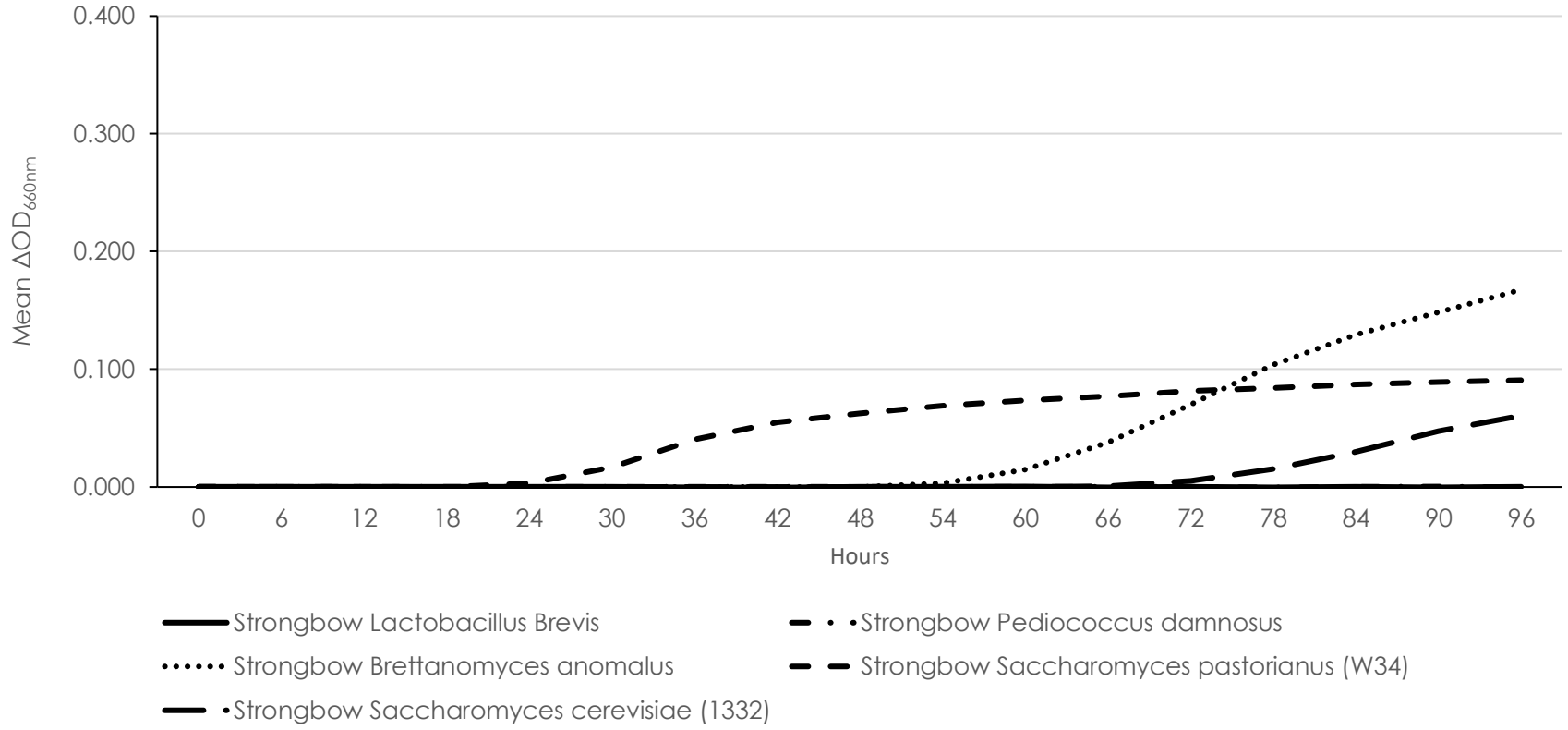
5830

5831 Growth curves of five different micro-organisms in Guinness beer, at 37°C for 96 hours.

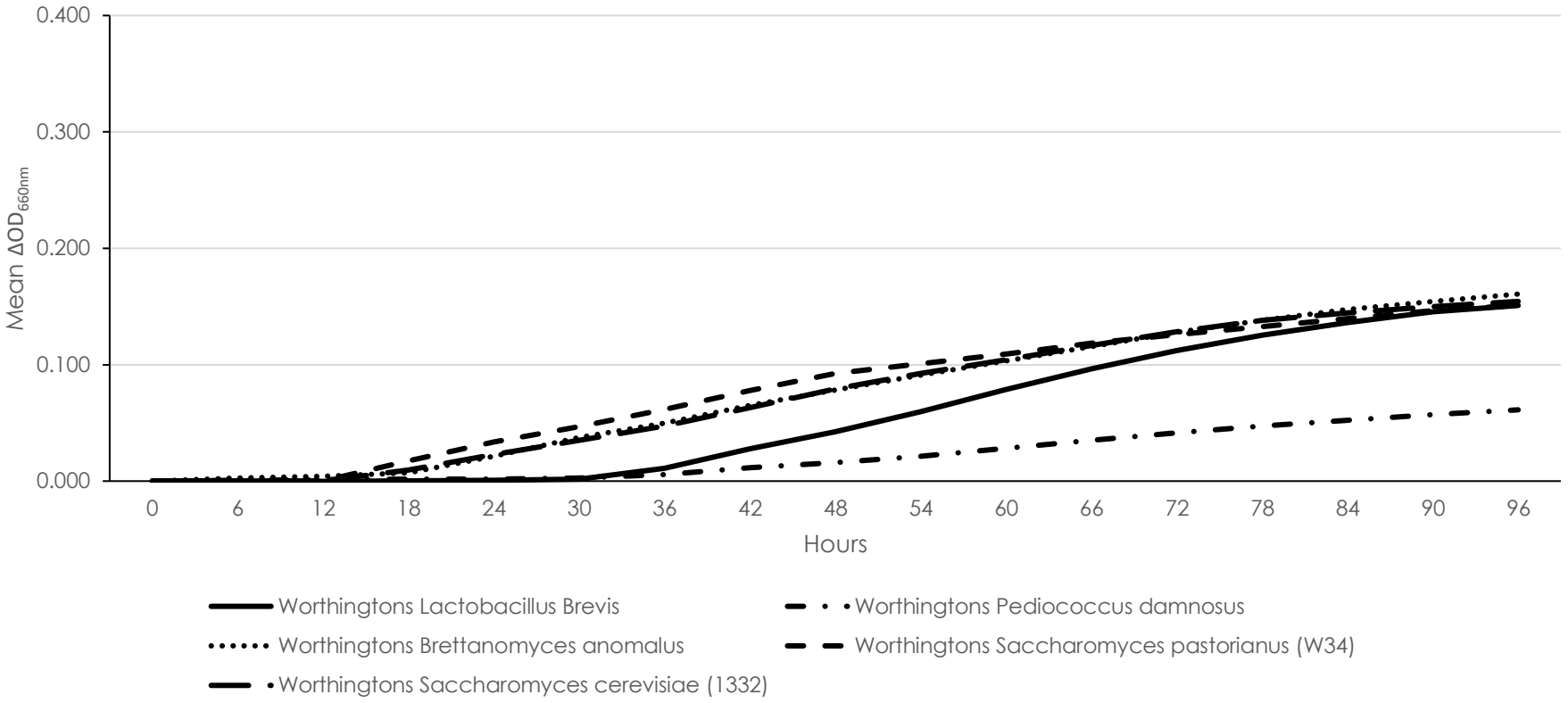


5832

5833 Growth curves of five different micro-organisms in Strongbow Dark Fruit cider, at 37°C for 96 hours.



Growth curves of five different micro-organisms in Strongbow cider, at 37°C for 96 hours.



Growth curves of five different micro-organisms in Worthington beer, at 37°C for 96 hours