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The spoilage of lager by draught beer microbiota

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Early view - before inclusion in issue 4

Abstract

Why was the work done: To determine whether the susceptibility of lager to microbiological spoilage is determined by composition, microbiota or both. To assess beer spoilage by a consortium of yeasts and bacteria from draught beer rather than pure laboratory cultures.
How was the work done: Four draught beer styles - cask ale and keg lager, ale, and stout – were sampled twice in five different public houses in four different locations. The beers were forced by static incubation at 30°C for four days. 'Challenge testing' with an inoculum of heterogeneous microorganisms from the forced samples was used to assess the spoilage of ten commercial lagers by the increase in turbidity at 660 nm. The same approach was used to evaluate the role of nutrients in beer spoilage by forcing with the addition of yeast extract or vitamins (thiamine and riboflavin).
What are the main findings: The ten lagers varied in susceptibility to spoilage ranging three-fold from the least to most spoilable. Average spoilage of the beers was comparable for microorganisms from lager, keg ale and stout but ca. 50% greater with microorganisms from cask ale. The ranking of spoilage of the 10 lagers was similar for microbiota from cask ale, keg ale and stout but less so from lager. Spoilage was influenced by beer composition and was inversely related to beer pH and level of free amino nitrogen. The addition of yeast extract stimulated spoilage of the least spoilable lager but the addition of vitamins B_1 and B_2 had little or no effect. Spoilage was extensive at 30°C, measurable at 12°C but imperceptible at 2°C.
Why is the work important: The oft-quoted statement that beer is 'robust to microbiological spoilage' is a fallacy. All ten lagers were spoilt by draught beer microorganisms, but some were more spoilable than others. It is suggested that spoilage may be reduced by lowering beer pH and curbing the availability of nutrients for microbial growth. Whilst (as would be expected) beer storage at 2°C suppresses microbial growth, storage at 12°C (as practiced in UK public house cellars) allows spoilage microorganisms to grow in beer. Although the threat of microbial spoilage in the brewing process is managed by good manufacturing practices, draught beer is vulnerable and requires more focus and commitment to hygienic practices to assure quality.

Keywords:

draught beer, spoilage, lager, composition, microbiota

Introduction

The microbiological spoilage of beer was recognised in the 1870s with Louis Pasteur observing the 'ferments of disease' in ale and porter whilst Horace Brown noted that the 'principal disease organisms of beer' included both bacteria and yeasts (Anderson 1989). Regrettably, despite the passage of time, beer spoilage by microorganisms continues to be a concern. Indeed, in recent years, there have been two books on brewing microbiology (Hill 2015; Bokulich and Bamforth 2017) together with reviews on beer spoilage (Rainbow 1981; Jesperson and Jakobsen 1996; Vaughan et al. 2005; Hill 2009; Quain 2015; Suzuki 2020; Kordialik-Bogacka 2022).

The symptoms of microbial spoilage are subjective, differing between consumers, and ranging from a nuance to a dramatic change in flavour, aroma or appearance. Some beer styles are more flavour/aroma forward and better hide the effects of microbial activity. However, some of the spoilage products formed by microorganisms – diacetyl, acetaldehyde, acetic acid, hydrogen sulphide - are more impactful at low concentrations, particularly in lighter, balanced beers. In turn, the spoilage characteristics reflect product composition and the microorganisms growing in the beer.

The signature microorganisms implicated in beer spoilage include aerotolerant gram-positive bacteria (*Lactobacillus, Pediococcus*), aerobic/microaerophillic gram-negative bacteria (*Acetobacter, Gluconobacter*), facultatively aerobic yeasts (*Saccharomyces*) and aerobic yeasts (*Brettanomyces, Candida, Pichia, Rhodotorula*) (Rainbow 1981). Ironically, initiatives to significantly reduce dissolved oxygen in packaged beer and improve flavour stability/shelf life have seen the emergence of obligate anaerobic gram-negative spoilage bacteria. Spoilage by the two genera – *Megasphaera* and *Pectinatus* – are notable for producing 'offensive off-flavours and aromas' (Ziola and Bergsveinson 2017).

The susceptibility to spoilage of foods is determined by four factors – intrinsic, extrinsic, processing and implicit (Lianou et al. 2016). 'Intrinsic factors' reflect the composition of the food which can be ill-defined and either support or inhibit the growth of microorganisms. 'Extrinsic factors' consider the environmental conditions experienced during production. These include storage at low temperature to restrict microbial growth and the use of inert gases to limit oxygen. Together, these extrinsic factors are considered more important than the intrinsic food matrix in minimising microbial growth (Lianou et al. 2016). 'Processing factors' include thermal treatments that impact on the loading of viable microorganisms in food. Finally, 'implicit factors' recognise the interactions between food and microbiota as well as between microorganisms.

For beer, there are diverse intrinsic factors that support the growth of contaminating microorganisms. Nutrients can be contributed from yeast autolysis (Rainbow 1952; Kulka 1953) or remain in beer post-fermentation. For example, *Saccharomyces* brewing yeasts are unable to utilise oligosaccharides or short chain glucose molecules (dextrins) which can be metabolised by diastatic strains of *Saccharomyces cerevisiae* (Andrews and Gilliland 1952) and *Brettanomyces bruxellensis* (Crauwels et al. 2017). Similarly proline, an abundant amino acid in wort and beer, is hardly assimilated by *S. cerevisiae* (Tanahashi et al. 2022) but can be used as a nitrogen source by *B. bruxellensis* (Crauwels et al. 2015). Conversely, there are numerous intrinsic factors that are considered to limit or slow the growth of spoilage microorganisms. These include antimicrobials – ethanol (0-8% ABV), hop compounds (17-55 mg iso-alpha acids/L), pH (3.8-4.7), negligible oxygen (< 100 µg/L), carbonation (ca. 0.5% w/v), sulphur dioxide (5-30 mg/L) (Jesperson and Jakobsen 1996) and organic acids (500 mg/L) (Coote and Kirsop 1974).

The extrinsic factors relevant to brewing include product cooling and minimisation of oxygen which have long been established across maturation, filtration, and the packaging of beer. It is noteworthy that the management of oxygen in brewing is driven by the needs of flavour stability rather than microbiology. Indeed, such conditions limit aerobic microorganisms but select for anaerobes which spoil beer. In process, microorganisms in beer are minimised through effective cleaning and hygienic practices with processing factors such as filtration, sterile filtration (Freeman 2015) or pasteurisation

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(Wray 2015) reducing the microbial load to commercially acceptable levels.

The role of implicit factors and interactions between microorganisms in beer spoilage and, in turn, beers are less well defined. Broadly, irrespective of the microbial mix, interactions can be synergistic or antagonistic (Huis in't Veld 1996). Positive interactions between microorganisms and within cell populations include mutualism with multi-metabolite 'cross feeding' such as between *Saccharomyces* yeast and lactic acid bacteria in liquid culture (Ponomarova et al. 2017) and in biofilms (Fan et al. 2020). Synergy can also be generic as observed during beer spoilage where the environment changes though cell metabolism or autolysis becoming more supportive to microorganisms which were initially incapable of growth (Kulka 1960). Conversely, for some organisms, such environmental changes can be antagonistic and supress growth.

Further, susceptible bacteria and yeast within heterogenous microbiota can be killed by toxins, such as the peptide nisin that kills *Lactobacillus* and other gram-positive bacteria when added to brewery fermentations (Ogden 1986). Similarly, 'killer' yeasts (*Saccharomyces, Candida, Pichia* and other genera) are ubiquitous in the environment and secrete proteins that kill sensitive yeasts by disrupting the plasma membrane (Marquina et al. 2002). Introduction of killer factor from *Saccharomyces* into brewing yeasts was successful in killing brewing strains and wild *Saccharomyces* yeast but had no impact on other yeast genera (Hammond and Eckersley 1984). Overlaid on this targeted antagonism, sub-populations of microorganisms can be selectively killed. For example, in *Saccharomyces* yeasts, cell death 'within populations can be because of the application of lethal external stresses but more usually is a programmed process in which targeted cells commit suicide in an expression of altruism' (Boulton 2021). A recent study in both fission and budding yeasts (Oda et al. 2022) reported - in response to glucose starvation - the release of low molecular weight autotoxins which kill latecomers into the environment.

A handful of studies have reported differences in the susceptibility of beers to spoilage. Cosbie (1943) working on spoilage by *Acetobacter*, reported that 'beers vary considerably in their resistance to infection and the organisms which apparently flourish with ease in one beer appear to be suppressed in another'. More recently, similar observations have been reported from challenge tests with *Lactobacillus* and *Pediococcus* species into 31 beers (ale, lager, and stout – Dolezil and Kirsop 1980), 17 lagers (Fernandez and Simpson 1995) and 10 beers (lager, wheat and pilsner – Geissler et al. 2016). The resistance of beers to spoilage by lactic acid bacteria has been tentatively attributed to a heat labile yeast metabolite (Dolezil and Kirsop 1980) and, in more detail, low pH and lower levels of free amino nitrogen and maltotriose (Fernandez and Simpson 1995).

The work reported here focusses on the microbial spoilage of lager which in 2019 (pre-pandemic) accounted for 64% of draught beer sales and 77% of total beer sales in the UK (British Beer and Pub Association 2021). Accordingly, the spoilage of 10 commercial lagers was assessed by challenge testing with microorganisms from four styles of draught beer sampled from 20 public houses on two occasions. The culture-dependent microbiota found in the four styles of draught beer are reported in a companion publication (Jevons and Quain 2022). In addition, the impact of nutrients on beer spoilage was evaluated by the addition of yeast extract and vitamins (thiamine and riboflavin) during forcing of two beers with the highest and lowest susceptibility to spoilage.

Materials and methods

Codes – beer and accounts

To provide continuity with previous studies (Mallett et al. 2018; Mallett and Quain 2019; Jevons and Quain 2021, 2022; Quain 2021), the beer brands and public houses used in this work are anonymised.

Draught beers

In the primary study, four draught beers were sampled post dispense. The four styles - standard lager (SL3), stout (ST1), cask ale (SC1) and keg ale (KA1) – are market leaders in their category and available nationally in the UK. The ethanol content as alcohol by volume (ABV) was 4% (SL3), 4.2%

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(ST1), 4.4% (SC1) and 3.6% (KA1), The four beer styles were sampled twice from five accounts in four different locations (Nottingham, Derby, Burton and three villages in Derbyshire) (Jevons and Quain 2022). Sampling was covert and, accordingly, line cleaning frequency and other hygienic practices were not known. Samples (250 mL) were collected in sterile Duran bottles, kept cold (4-6°C) and processed using the forcing test.

Forcing test

The microbiological loading of draught beer was determined by incubating the beer at 30°C for 96 hours and measuring the increase in absorbance at 660 nm (Mallett et al. 2018). Cycloheximide (4 mg/L) was added to samples of unpasteurised cask beer to suppress the growth of indigenous brewing yeast. Samples were assessed in triplicate. The increase in absorbance reflected the microbiological quality of the beer and was categorised as A/excellent (Δ A_{660} 0-<0.3), B/acceptable (Δ A_{660} 0.3-<0.6), C/poor (Δ A_{660} 0.6-<0.9) and D/unacceptable (Δ A_{660} >0.9).

Quality index

Forcing retrospectively reflects the microbiological quality of beer at dispense. A cumulative 'quality index' enables the tracking over time of individual brands or collectively an account.

The quality index is 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 (Mallett et al. 2018; Mallett and Quain 2019; Jevons and Quain 2022).

Packaged lagers

The ten lager brands packaged (in bottle or can) were categorised by ABV (as 'standard lager' (SL) ≤ 4.1% ABV and > 4.1% ABV for 'premium lager' (PL). Of the beers, four were 'standard lager' (SL1,3,5 and 6) and six beers were 'premium lager' (PL1,2,3,6,8 and 9).

Challenge test

Spoilage by the microbiota from 40 samples of draught beer (10 samples per style) was determined post forcing by inoculating the microorganisms at a fixed dilution into the 10 commercial lagers and then forcing them at 30°C for 96 hours. To standardise the method, an aliquot of beer ex-forcing equivalent to A_{660} = 1 (e.g., 4 mL at Δ A_{660} = 0.25, 2 mL at Δ A_{660} = 0.5) 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 10 brands of lager (25 mL pasteurised beer ex can or bottle in 30ml universal plastic bottles) in duplicate, forced at 30°C for 96 h and the Δ A_{660} determined.

Supplementation with yeast extract

Two draught beers (stout ST1 and lager SL3) were sampled from two accounts (V6 and V15) in September 2019. After forcing, the spoilt beers were inoculated (as above, A_{660} = 0.02) into PL8 and PL9 (25 mL), in duplicate, supplemented with yeast extract at 50, 200 and 500 mg/L. After incubation for 96 hours at 30°C, the Δ A_{660} was determined. The experiment was repeated with the same forced samples after nine days storage at 4°C. The impact of yeast extract was the same whether the stock solution was autoclaved or sterile filtered.

Supplementation with vitamins

Draught beers (stout ST1 and lager SL3) were sampled from account V5 in October 2019. After forcing, microorganisms were inoculated (A_{660} = 0.02) into lagers PL8 and PL9 (25 mL, in duplicate) supplemented with yeast extract (50, 200 and 500 mg/L), riboflavin (vitamin B_2) (150, 300, 600 and 1500 µg/L) or thiamine (vitamin B_1) (50, 100, 200 and 500 µg/L). After incubation for 96 hours at 30°C, the Δ A_{660} was determined. Stock solutions of riboflavin and thiamine were sterile filtered.

Spoilage and incubation temperature

Draught beers (lagers PL3 and SL6) were sampled on three occasions from account L7 in September and October 2017. The beers were forced at 2, 12 and 30°C and the Δ A_{660} measured for four and eight days.

Beer analysis

The 10 lagers were analysed for present gravity (PG) and ABV (Anton Paar DMA 4500 Acolyzer Plus),

colour at 430 nm (Shellhammer 2009) and pH (Jenway 3510 pH meter). Bitterness was determined using ASBC Beer-23A (ASBC 2009). Free amino nitrogen (FAN) was measured in degassed beer using ASBC Wort -12 (ASBC 2010). Total acidity was determined by titration with sodium hydroxide (ASBC 2016). Glycerol was determined enzymically (K-GCROLGK, www.megazyme.com).

Statistics

Principal Components Analysis and ANOVA was performed using XLSTAT (www.xlstat.com). MS Excel was used for other data analyses.

Results and discussion

Draught beer quality

The four draught beer styles – keg lager, stout, cask ale and keg ale – were sampled twice in five different accounts in four different locations. The microbiological quality of the beer samples was assessed by forcing. Cask ale was of the best quality as determined by the quality index (90%) with beers being either 'excellent' or 'acceptable'. Both lager and stout samples had a quality index of 75%, with keg ale showing a quality index of 67.5% with samples ranging from 'acceptable' to 'poor' (Jevons and Quain 2022).

Spoilage by draught beer microbiota

In all, 40 draught beer samples – 10 for each beer style - were obtained from 20 different accounts. The indigenous microorganisms were amplified by forcing at 30°C with a standard inocula (A_{660} = 0.02) added to 10 lager brands. The beers were forced and the increase in absorbance determined. Table 1 details the spoilage metrics for the challenge tests and associated statistics for each beer style by account. The average spoilage (Δ_{660}, 100 samples per style) was 0.39 for microorganisms from lager and keg ale, 0.44 from stout and 0.61 from cask beer.

Samples of each beer style were taken from each account on two occasions four or more weeks apart. The results from each pair of 50 challenge tests were subject to Principal Components Analysis (Figure 1) and all four beer styles were differentiated from each other as clusters. Linear regression analysis showed a moderate correlation (R^2 = 0.51-0.55) between the two sets of data for microorganisms from lager, stout and cask beer but without correlation for keg ale.

Table 1.

Challenge testing of 10 lagers. Spoilage (as absorbance) by microorganisms sampled on two occasions from each public house.

Lager (SL3) - villages					
Account	B7	V5	V6	V8	V13
Mean	0.480	0.398	0.338	0.264	0.478
Std Dev	0.122	0.112	0.112	0.064	0.131
CV (%)	25	28	33	24	27
Mean of five accounts ± SEM = 0.392 ± 0.055					

Stout (ST1) - Nottingham					
Account	N5	N8	N14	N15	N16
Mean	0.45	0.359	0.401	0.546	0.461
Std Dev	0.177	0.133	0.177	0.168	0.198
CV (%)	39	37	44	31	42
Mean of five accounts ± SEM = 0.443 ± 0.050					

Ale (KA1) - Derby					
Account	D2	DE10	D13	DE19	D20
Mean	0.347	0.394	0.405	0.378	0.408
Std Dev	0.136	0.132	0.117	0.144	0.16
CV (%)	39	34	29	38	39
Mean of five accounts ± SEM = 0.386 ± 0.019					

Cask ale (SC1) - Burton					
Account	B2	B10	B11	B12	B13
Mean	0.660	0.543	0.603	0.633	0.586
Std Dev	0.187	0.146	0.226	0.177	0.146
CV (%)	28	27	35	28	25
Mean of five accounts ± SEM = 0.605 ± 0.033					

Further comparative analysis (Table 2, Figure 2) of spoilage of the individual lager brands shows that the susceptibility to spoilage varied. Ranking of spoilage using a standard inoculum of microorganisms from 40 samples of forced draught beer per brand showed premium lager PL8 to be three-fold more spoilable than PL9. Ranking of spoilage was broadly aligned with microbiota from stout, keg ale and cask ale. However, spoilage by lager microorganisms was less predictable, with PL6 the most spoilable although PL9 remained the least.

Beer composition and spoilage

The 10 lagers used to assess spoilage were mainstream UK lagers produced by global brewing companies. Analysis (Table 3) showed the beers to be broadly similar, with small differences in present

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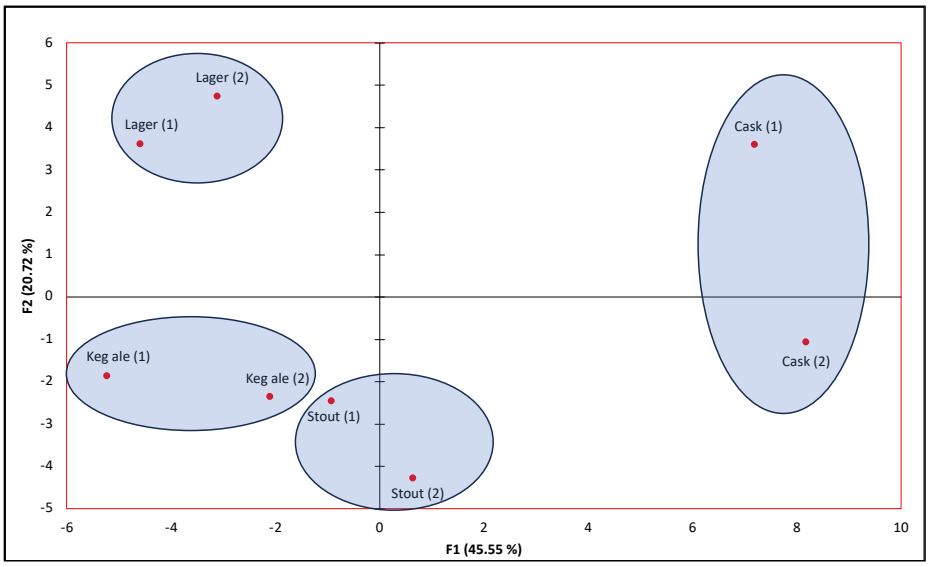

Figure 1.

PCA plot of the spoilage of 10 lagers by paired samples from 20 public houses. Samples were obtained 4-6 weeks apart, forced and the microbiota inoculated into the lagers (as described in 'challenge testing').

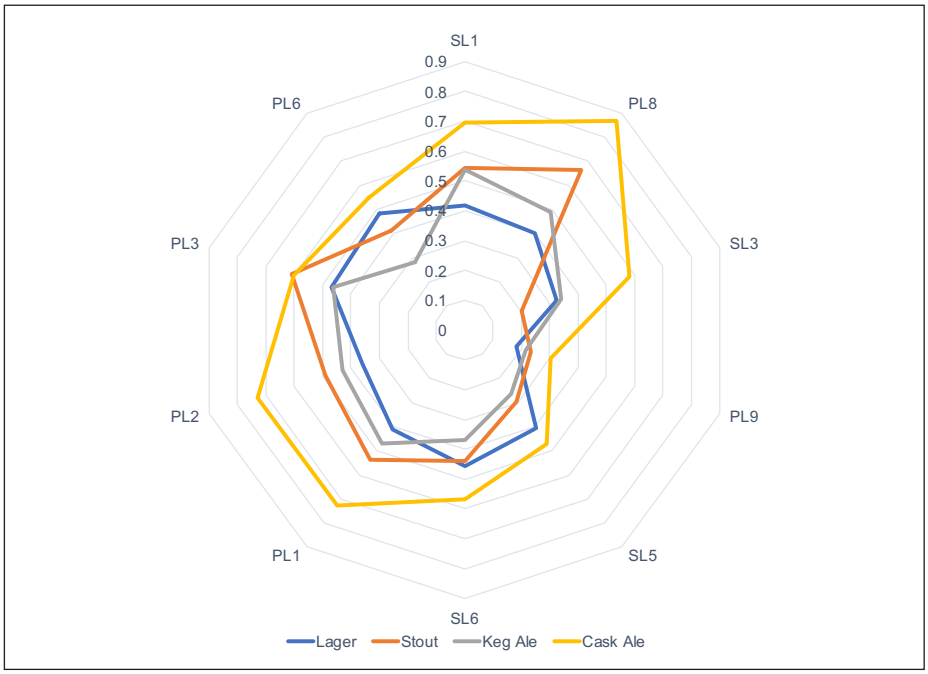

Figure 2.

Radar plot of the spoilage of 10 lagers by microorganisms from lager, stout, keg and cask ale. Each data point represents the mean Δ_{660} from challenge tests in duplicate with microbiota from 10 samples of each beer style obtained on two occasions from five public houses.

	Spoilage capability (10 = high, 1 = low)				Spoilage	
Lager screen	Lager SL3	Stout ST1	Ale KA1	Cask ale SC1	Mean[a]	Mean[b] Δ_{660}
SL1	7	8	10	7	9	0.549
SL3	2	1	4	5	2	0.363
SL5	5	3	2	2	2	0.360
SL6	8	5	5	4	5	0.458
PL1	6	7	8	8	7	0.537
PL2	3	6	6	9	6	0.502
PL3	9	9	7	6	8	0.535
PL6	10	4	3	3	4	0.432
PL8	4	10	9	10	10	0.606
PL9	1	2	1	1	1	0.235

Table 2.

Ranked spoilage of lagers with draught beer microbiota.

[a] Average ranking for spoilage capability
[b] from 40 individual measurements of spoilage (10 for each microbiota)

Figure 3.

Spoilage of 10 lagers v product composition (pH and FAN). Data for mean spoilage reported in Table 1 against product composition in Table 2. Relationship between pH (blue, $R^2 = 0.537$) and FAN (orange, $R^2 = 0.538$) v spoilage.

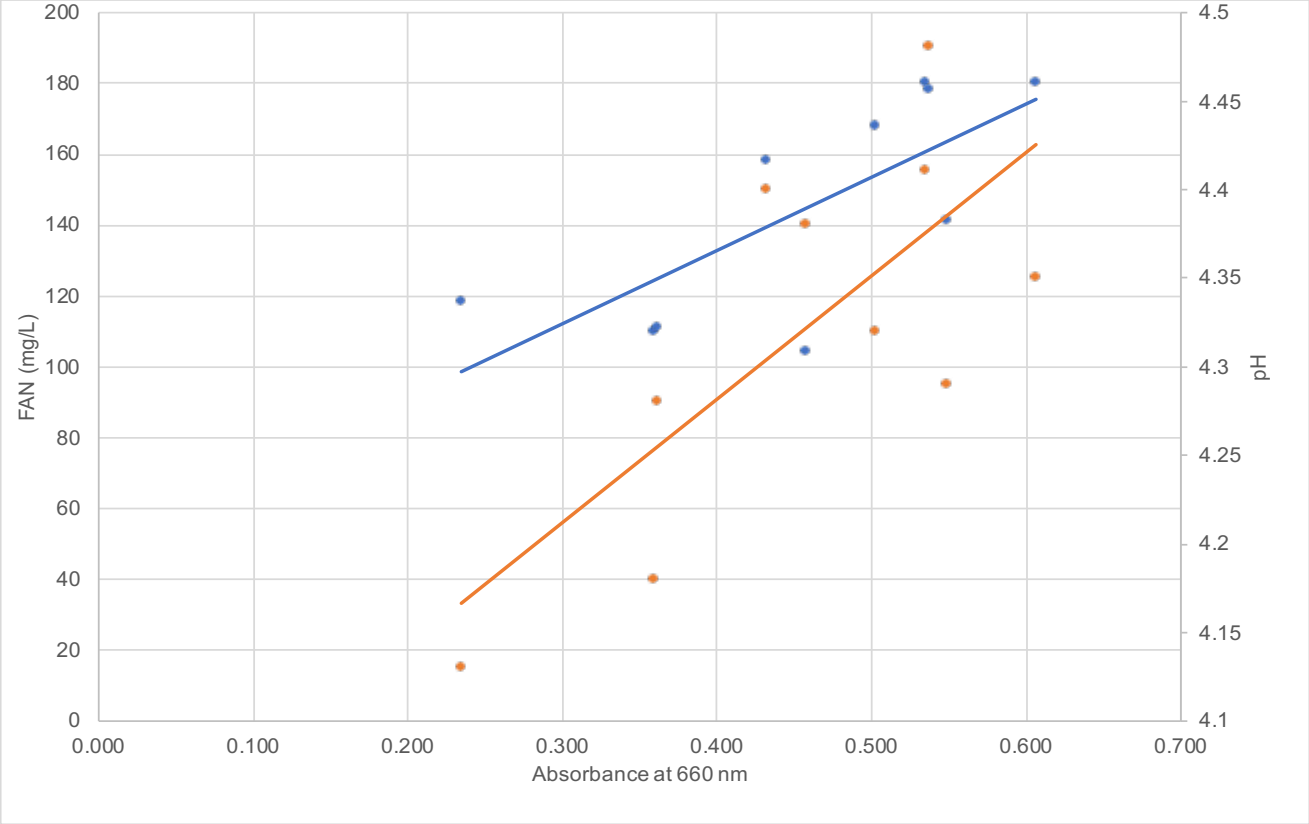

Table 3.

Analysis of 10 lagers.

Lager	ABV	Present Gravity (g/mL)	pH	Total acidity	Colour	Glycerol (g/L)	IBU[b]	FAN[c] (mg/L)	Ingredients*
SL1	4.04/4.1	1002.6	4.29	30.6	4.1	1.08	9.2	141	1
SL3	4/4	1005.2	4.28	26.6	4.5	1.06	8.9	111	2,3
SL5	4.04/4	1006.0	4.18	24.1	3	1.21	4.6	110	2,3
SL6	3.91/4	1007.3	4.38	22.5	5.2	1.2	9.2	104	1,4
PL1	4.97/5	1006.8	4.48	27.1	4.8	1.37	9.6	178	1
PL2	5.17/5.1	1006.8	4.32	23.3	4.4	1.26	14.6	168	1,5
PL3	4.85/5	1009.7	4.41	27.7	4.6	1.44	12.8	180	1
PL6	4.7/4.8	1007.2	4.40	27.0	4	1.4	14.3	158	1,5
PL8	4.41/4.5	1007.8	4.35	19.5	3.7	1.12	4.6	180	1,6
PL9	4.78/4.5	1006.6	4.13	24.5	5.5	1.41	12.2	118	1,5

[a] alcohol by volume – by analysis/declared
[b] international bitterness units
[c] free amino nitrogen

* As reported on can or bottle packaging, 1 = malted barley, barley malt; 2 = barley; 3 = wheat; 4 = glucose syrup; 5 = maize; 6 = rice

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Figure 4.

Impact of the addition of yeast extract on beer spoilage. Microbiota from lager (SL3) and stout (ST1) from two accounts (V6, V15) were inoculated into lagers PL8 and PL9 supplemented with yeast extract (50, 200 and 500 mg/L).

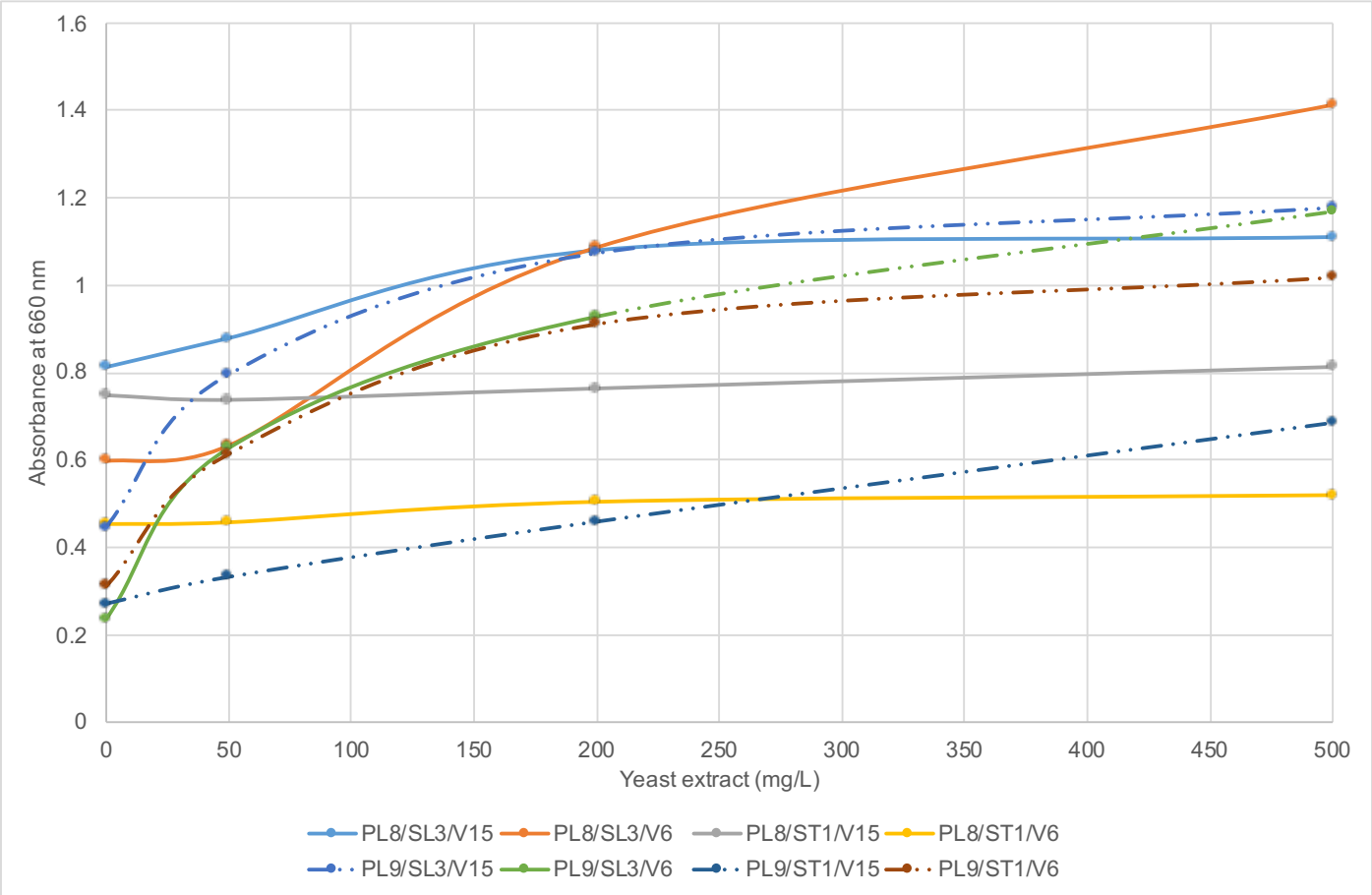


Table 4.

Addition of B vitamins and impact on spoilage.

Inoculum	Beer	none	Supplementation		
			Yeast extract[1]	Riboflavin[2]	Thiamine[3]
		A_{660}	fold change	fold change	fold change
SL3	PL8	0.454	2.4	1.1	1.3
SL3	PL9	0.325	3.3	1.4	1.4
ST1	PL8	0.728	1.5	1	1
ST1	PL9	0.374	2.8	1.1	1

[1]. yeast extract at 500 mg/L
[2]. riboflavin at 1500 µg/L
[3]. thiamine at 500 µg/L

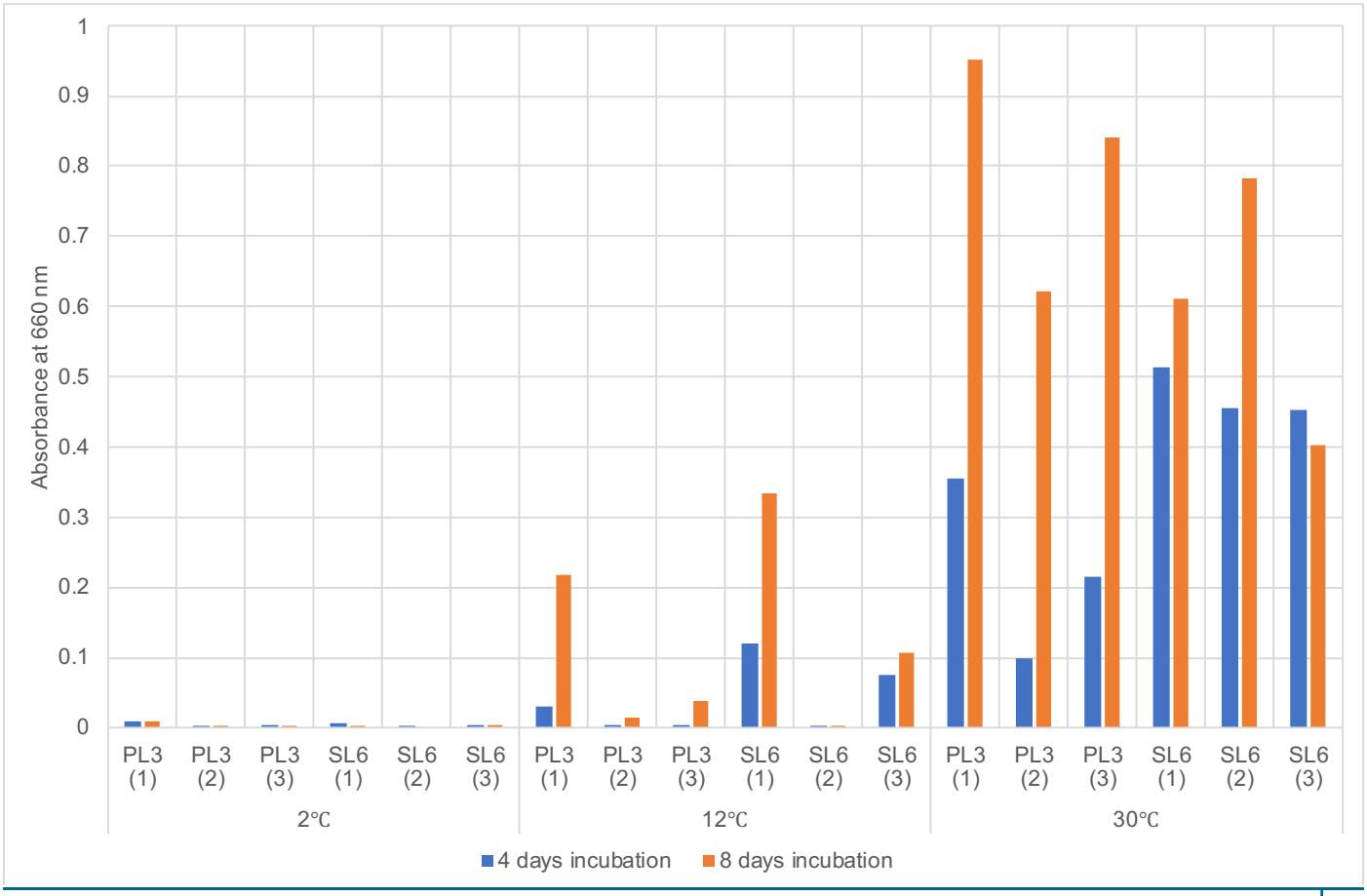
gravity, pH, colour, free amino nitrogen (FAN) and bitterness (IBU). The ethanol content (ABV) divides the 10 lagers into two groups - 'standard' (≤ 4.1% ABV) or 'premium' (> 4.1% ABV). There was no relationship between spoilage and ABV or other parameters (PG, colour, bitterness, glycerol). However, beer spoilage was directionally related (with moderate correlation) to pH and free amino nitrogen (FAN) (Figure 3). A plot (data not shown) of all three parameters resolved two clusters without overlap of least spoilable (SL3,5,6; PL6,9) and more spoilable lagers (PL1,2,3,8; SL1). A one-way ANOVA was performed to compare the effect of pH and FAN on spoilage. There was no statistically significant difference between them (both $p = 0.016$).

Beer composition is determined by raw materials and their processing from brewhouse to final package. Whether nutritional differences influence beer spoilage was evaluated by adding yeast extract to the least (PL9) and most spoilable lager (PL8) and assessing the impact on microbial growth using challenge testing. Microorganisms from lager (SL3) and stout (ST1) from two accounts (V6, V15) were inoculated into lagers PL8 and PL9 supplemented with yeast extract (50, 200 and 500 mg/L). Figure 4 shows that yeast extract stimulated the spoilage of the 'least spoilable' lager (PL9) by 3.3-fold (range 2.4-4.9). Conversely, the addition of yeast extract had less impact on the spoilage of the 'most spoilable' lager (PL8) where microbial growth increased 1.9 ± 0.5 fold (lager SL3) and 1.2 ± 0.1 fold with inocula from stout (ST1).

The effect of supplementation of vitamins - thiamine (B_1) and riboflavin (B_2) – on the spoilage of lagers PL8 and PL9 was investigated with microorganisms from stout (ST1) and standard lager (SL3) (Table 4). The addition of riboflavin or thiamine to lager PL8 had no effect on the growth of microorganisms from stout ST1. Both vitamins stimulated the growth of microorganisms from SL3 in PL8 and PL9 together with ST1 in PL9. Stimulation from the highest addition of thiamine (500 µg/L) and riboflavin (1500 µg/L) was limited and much less than with yeast extract which was comparable to the experiments

Figure 5.

Impact of forcing temperature and time on microbial spoilage. Lagers PL3 and SL6 were sampled from on three occasions from account L7 and forced at 2, 12 and 30°C for four and eight days.



reported in Figure 4. Predictably, the incubation temperature had a significant effect on the spoilage of forced draught beer samples (Figure 5). At 2°C, changes in ΔA_{660} were barely measurable with an average change of 0.004 after eight days. At 12°C, the ΔA_{660} was 0.040 and 0.119 after four and eight days. At 30°C – the temperature used for the forcing method – the ΔA_{660} for the same samples was 0.349 after four days and 0.702 after eight days.

Discussion

The trade samples of draught beer were of varying quality (Jevons and Quain, 2022). The ten cask beer samples were either 'excellent' or 'acceptable' reflecting the need for superior cellar management skills to stock this style. The quality of lager ('excellent' to 'poor') and stout ('excellent' to 'unacceptable') was more variable. Of the four styles, keg ale quality was the poorest ranging from 'acceptable' to 'poor'. It is noteworthy that the aggregated 'quality index' for lager SL3 (75 v 84%) and for keg ale KA1 (67.5 v 68.3%) was similar that reported previously in a larger survey of trade quality (Mallett and Quain 2019).

Using Principal Coordinates Analysis to compare the two rounds of challenge testing of the ten lagers with microbiota from the four beer styles revealed four independent clusters (Figure 1). F1 and F2 accounted for 66% of the variation with the separation on the x-axis average spoilage by beer style reflecting lager, stout, keg ale and cask ale (Figure 1). From this, the degree of spoilage was consistent over time suggesting the microbial population in the dispene lines to be qualitatively constant. The companion publication (Jevons and Quain 2022) reported the culture-based microbiota of the inocula used in this work with 28 different microorganisms identified, and *Brettanomyces bruxellensis*, *B. anomalus* and *Acetobacter fabarum* predominating in all four beer styles. The argument that the microbiota is consistent was supported by the recovery of specific microorganisms in both samples from an account on 49 occasions.

Challenge testing with microorganisms from draught beer resulted in the variable spoilage of the commercial lagers (Figure 2) with lager PL8 spoiling the most and lager PL9 the least. Microbiota from cask beer resulted in the enhanced spoilage of all beers. The ranking of spoilage (Table 2) by microorganisms from stout, keg ale and cask ale were similar (average variation from the mean = 0.9). However, the order of spoilage of the 10 beers by lager microbiota was less well aligned (average variation from the mean = 2.5), possibly as the microorganisms was selected for growth in lager. Indeed, the microbiota found in lager (SL3) from the five accounts sampled on two separate occasions was distinct from that in stout, ale and cask ale containing less yeast and more lactic acid bacteria (Jevons and Quain 2022).

The differing spoilage of lagers PL8 and PL9 when challenge tested with draught beer microorganisms has been found consistently over three or more years, and has included the change of ABV of PL9 from 4.8 to 4.5%. Comparable results for the spoilage of PL8 and PL9 have been reported in another study of the vulnerability to spoilage of alcohol-free and low alcohol beers (Quain 2021).

Both low pH and low free amino nitrogen (FAN) are intrinsic factors that would be anticipated to slow or limit the growth of spoilage microorganisms. A study of lager composition and resistance to spoilage by lactic acid bacteria (Fernandez and Simpson 1995) reported correlation coefficients (R) for FAN (-0.59) and pH (-0.72). Similarly, the susceptibility to spoilage by draught beer microbiota of several alcohol-free beers (Quain 2021) was inversely related to beers with the lowest pH and FAN. Here, beer spoilage by challenge testing was broadly related to beer pH and free amino nitrogen (FAN) (Figure 3). Directionally, spoilage was enhanced in brands with a higher pH and level of FAN.

In practice, beer ph ranges from about 3.9 to 4.6 and – even acidic beers - will only slow microbial growth but not stop it. Lambic, the Belgian sour beer is spontaneously fermented by many of the same microorganisms involved in the spoilage of draught beer (Jevons and Quain 2022). The production of Lambic takes three years with a succession of microbiota over which time the pH drops from 4.5/5 to 3/3.5 (Van Oevelen et al. 1977: Bongaerts et al. 2021). As acetic acid and lactic acid bacteria together with *Brettanomyces* yeasts grow at pH 3/3.5, this suggests that beer spoilage is not overly compromised by a product pH of 4. Indeed, draught ciders can be microbiologically spoilt and

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support biofilm growth (Jevons and Quain 2021) at a pH range of 3.25-3.65 (Qin et al. 2018).

The growth of draught beer microbiota in minimal media containing maltose, ethanol and yeast extract was stimulated when the concentration of yeast extract was increased from 50 to 250 mg/L (Quain 2021). In Figure 4, the addition of yeast extract stimulated the growth of microorganisms from lager (SL3) and (to a lesser extent) stout (ST1) in lager PL8 and 9. The stimulation of growth in the least spoilable PL9 was almost three-fold greater than with lager PL8. As yeast extract contains B vitamins, amino acids, peptides, carbohydrates and ions, supplementation presumably meets some of the nutritional deficiencies in both beers but particularly so in lager PL9. Analysis (Table 3) shows PL9 to have a lower level (118 mg/L) of FAN (amino acids, peptides and ammonium ions) compared to PL8 (180 mg/L).

Work by Hucker et al (2017) reported that the addition of thiamine (vitamin B_1) or riboflavin (vitamin B_2) enhanced the growth in minimal media of spoilage microorganisms, *Levilactobacillus brevis* (*Lactobacillus brevis*), *Pedicoccus damnosus*, *Acetobacter aceti* and two *Brettanomyces* yeasts. Here, the impact of either B vitamin during challenge testing (Table 4) with draught beer microbiota was limited and only effective at the highest tested concentrations (500 µg/L thiamine, 1500 µg/L riboflavin) in supporting a small uplift in growth of the microorganisms from lager (SL3) rather than stout (ST1). Supplementation with yeast extract was more impactful suggesting that riboflavin and thiamine were unlikely to be limiting in PL 8 and 9. Lager has been reported (Hucker et al. 2011) to contain 35 µg/L thiamine and ca. 300 µg/L riboflavin.

The significance of nutrient deficiency in limiting beer spoilage has perhaps been overstated, as Rainbow (1952) noted that 'impoverishment is relative and appreciable concentrations of bacterial nutrients remain in finished beers'. Accordingly, microbial spoilage is determined by how supportive (or not) beer is as a medium in meeting the nutritional requirements of contaminating microorganisms. This will vary, as for example, brewery lactobacilli are more fastidious in their nutritional needs than acetic acid bacteria (Rainbow 1952). However, with at least 800 different compounds reported in beer (Cortacero-Ramırez 2003; Buiatti 2009), there is diverse mix of carbohydrates, amino acids, vitamins, nucleic acid derivatives and inorganic compounds to support the growth of microorganisms. These intrinsic factors will vary within and between beer styles reflecting the impact of raw materials and processing in the production of beer. Some factors are more subtle, for example the contribution of nutrients from yeast autolysis (Rainbow 1952; Kulka 1953) will be influenced by factors in fermentation and maturation and whether (or not) these processes are optimised for best practice to minimise autolysis.

The forcing method (Mallett et al. 2018) has been used either directly with draught beer or in challenge testing to assess the spoilage of beers. The method enables comparison of the growth of heterogeneous 'environmental' microorganisms after four days incubation at 30°C. The method is 'directional' in retrospectively providing a measure of microbial loading or the capacity of inocula to grow (Mallett and Quain 2019; Jevons and Quain 2021, 2022; Quain 2021). The method is geared to practicality with incubation at 30°C which - over four days - accelerates the growth of microorganisms.

The mixed microbiota found in draught beer (Jevons and Quain 2022) are likely to be mesophiles growing at an optimum temperature of 30-40°C and a minimum temperature of 5-15°C. Accordingly, in Figure 5 two draught lagers (SL6, PL3) were sampled on three separate occasions and forced at 2, 12 and 30°C for four and eight days. Little or no growth was found at 2°C in keeping with beer storage temperatures in process and cellar temperatures for draught beer in the USA. Forcing at 12°C resulted in variable but significant growth of some of the draught beer samples. This is a concern as draught beer in the UK is stored in cellars at 12°C for a week or more and the dispense process results in microorganisms in the line reseeding beer in the keg (James Mallett and David Quain, unpublished results). As would be expected at 30°C, all the samples exhibited growth. Whilst an optimum temperature for mesophilic microorganisms, the possibility of spoilage at 'room temperature' is limited to products packaged in can or bottle. Thankfully, such events are increasingly rare as the majority of smallpack products are pasteurised.

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Conclusions

Microbiological spoilage of beer has long been recognised; its parameters have been defined but subject to relatively little study. This work extends the reports of Cosbie (1943) and others (Dolezil and Kirsop 1980; Fernandez and Simpson 1995; Geissler et al. 2016) that beers vary in their susceptibility to spoilage. Here, challenge testing of commercial lagers showed that the extent of spoilage was brand specific but was consistent with draught beer microorganisms from different styles.

The susceptibility to spoilage was related to composition, with beers with a lower pH and FAN exhibiting least spoilage. However, resistance to spoilage was overcome by nutrient supplementation with yeast extract. The nutritional status of beer is determined by raw materials with variations arising from inconsistent processing. Arguably, reducing product pH and minimising yeast autolysis during fermentation and maturation will increase the robustness of beer to spoilage by microorganisms.

In the brewing process, opportunities for spoilage are minimised by the application of good hygienic practices and 'extrinsic factors' (chilled storage). In bottle or can, low levels of microorganisms in beer are eliminated by 'processing factors' such as pasteurisation and sterile filtration. Product recalls due to microbiological spoilage are rare but are high profile and typically involve unpasteurised beers. Ironically, the spoilage of draught beer is all too common an experience for consumers, brand owners and retailers. This further highlights the importance of implementing regular and effective hygienic practices to assure the quality of draught beer. The microbiological spoilage of beer at 12°C is further justification for reducing the temperature of keg storage in cellars.

Author contributions

David Quain - methodology, conceptualisation, validation, investigation, writing (original draft).

Alexander Jevons – investigation, writing (review and editing).

Conflict of interest

Although Editor in Chief of the *Journal of the Institute of Brewing*, after submission of the manuscript, David Quain had neither involvement or visibility of the subsequent process including selecting reviewers, peer review and associated decision making. This was kindly facilitated by guest Editor, Professor Brian Gibson.

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