Research article

Received: 12 February 2018 Revised: 10 June 2018 Accepted: 11 June 2018 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/jib.520

Challenging the assumptions around the pasteurisation requirements of beer spoilage bacteria

Grzegorz Rachon,* Christopher J. Rice, Karin Pawlowsky and Christopher P. Raleigh

Current recommendations for beer pasteurisation are based on the study in 1951 by Del Vecchio and coworkers. In this work, 14 beer spoilage bacteria were screened for their ability to grow or survive in ale and stout together with the determination of their thermo tolerance at 60°C. Using a capillary tube method, the *D*-value (decimal reduction time) and *z*-value (temperature resistance coefficient) of the three thermo tolerant bacteria (*Acetobacter pasteurianus*, *Lactobacillus brevis* and *Lactobacillus hilgardii*) were determined. Validation of pasteurisation at a range of pasteurisation units (PU) in packaged product were performed in a tunnel pasteuriser. This study showed that eight of the 14 microorganisms were able to grow in both beer styles, whilst different thermo tolerances were observed amongst the spoilage bacteria. Effective pasteurisation of the selected microorganisms was achieved at significantly lower PU values than those recommended by the European Brewery Convention *Manual of Good Practice*. In package pasteurisation conducted at 1.6 PU resulted in greater than an 8-log reduction in viable cell numbers, resulting in 'commercial sterility'. Although this study demonstrated that successful pasteurisation was achieved for vegetative cells at significantly lower PU values than those recommended, further studies are required to demonstrate the optimal level of pasteurisation for spore forming bacteria and for yeast. © 2018 The Institute of Brewing & Distilling

Keywords: pasteurisation; spoilage bacteria; validation; capillary tubes; heat inactivation

Introduction

Contamination of beer is an important problem for the brewing industry, necessitating sterile filtration or heat treatment to maintain the biological integrity of the product. Approximately 70% of beer spoilage cases result from contamination by species of *Lactobacillus* and *Pediococcus (1)*, while *Lactobacillus brevis* has been reported as the most prevalent spoilage organism, regardless of the beer style investigated *(2)*. In addition, there are other aerobic and anaerobic beer and brewery-related spoilage organisms that represent a potential source of contamination in the brewing environment and where beer is served in a draught dispensing system, such as acetic acid producing bacteria (e.g. *Acetobacter* spp.), *Zymomonas* spp., *Megasphaera* spp., *Kocuria* spp. and *Pectinatus* spp. *(3,4)*. A number of yeast species can also spoil beer; however, this study focused on bacteria. It is anticipated that futher work will study the heat resistance of spoilage bacteria in biofilms and yeast ascospores. To combat these contaminants, pasteurisation is widely employed in the brewing industry; however the effectiveness of the process varies depending on the processing time and temperature, product composition and the type of contaminating organisms present *(5)*. Thermo tolerant bacteria and yeast are able to tolerate standard heat treatment regimes. Therefore, selecting the most suitable time–temperature pasteurisation regime for a particular product is not always straightforward. The brewing industry currently bases its pasteurisation regimes on long-established microbiological parameters: *D*- and *z*-value, and pasteurisation units (PU) of an organism under certain conditions *(6)*. The *D*-value is the time required at a specific temperature for a decimal (i.e. 1 log or 90%) reduction in the population of a microorganism; the *z*-value is defined as the change in temperature required for a 10-fold change in the *D*-value. The European Brewery Convention (EBC) *Manual of Good Practice (6)* provides basic recommendations for pasteurisation of a range of beer styles (Table 1), but it is suggested that the stated heat loads are over-estimated, resulting in over processing of the beverages, which may result in damage to aroma and flavour compounds *(7)*.

These EBC recommendations are based on a historical study, conducted by Del Vecchio *et al.* in 1951 *(8)*. Despite a number of concerns *(9–11)* associated with using Del Vecchio's *z*-value (6.94°C), it is still widely used. McCaig *et al. (12)* reported the failure of a flash pasteurisation at 26.5 PU owing to the presence of an *L. brevis* strain with a *z*-value >6.94°C. Similarly, Tsang and Ingledew *(13)* and Molzahn *et al. (14)* reported higher *z*-values for common beer spoilage bacteria. The Del Vecchio study includes several curious aspects that do not reflect the real world beer environment. For example, the authors used a completely fermented beer, supplemented with 5% boiled wort, thereby increasing the overall sugar concentration in the product. In addition, the authors inoculated the product with a cocktail of beer spoilage organisms (bacteria and yeast), basing the death kinetics on the most thermotolerant organism, an unidentified (and uncharacterised) 'abnormal yeast'. Crucially, this early work was never intended to provide a catch-all model for pasteurisation. Indeed, the authors themselves reported in the study that 'It should not be concluded that

* Correspondence to: Grzegorz Rachon, Campden BRI, Centenary Hall, Coopers Hill Road, Nutfield, Surrey RH1 4HY, UK. E-mail: grzegorz.rachon@campdenbri.co.uk

Campden BRI, Centenary Hall, Coopers Hill Road, Nutfield, Surrey RH1 4HY, UK

Table 1. Range of pasteurisation units for beers – EBC recommendations *(6)*

Product	Typical minimum PU	Typical maximum PU
Pilsner and lager beer	15	25
Ale and stout	20	35
Low alcohol beer	40	60
Non-alcoholic beer	80	120

the resistance found for the organisms in these tests are necessarily the maximum existing in the beer industry. Only numerous tests with different types and strains and on different beers and ales can determine this point' *(8)*. However, since its publication, the Del Vecchio study has been widely interpreted as providing an indicator for pasteurisation and its findings have been applied to a variety of beer styles containing a broad range of chemical parameters, without the robust microbiological data required to guarantee the microbial integrity of the product. In response to this uncertainty, many brewers have altered their processes to increase the level of pasteurisation to kill off any contaminants *(15)*, providing microbiological stability but risking damage to flavour compounds *(7)* and increasing the cost of the process *(16)*. Therefore, optimising pasteurisation for different beer styles and microorganisms is likely to result in reduced costs, lower energy utilisation and decreased water utilisation for breweries. However, to reap these benefits, a detailed study is required to provide evidence backed support for the brewing industry. Existing thermal survival data for these microorganisms is scarce *(17)*, with *Lactobacillus* spp. the best characterised bacteria *(18)*. From the few published studies, it is clear that a wide range of thermo tolerances exist, even within the same type of organism. For example, *Lactobacillus D*-values at 60°C (D_{60}) in beer have been reported which range from 0.77 to 3.70 min when performed in laboratory scale pasteurisation experiments *(13)*. However, data from a previously performed study at Campden BRI demonstrated considerably lower D_{60} values of 0.06–0.10 min for a strain of *L. brevis* (unpublished observations). Studies conducted by Adams *et al. (19)* highlighted the impact of the chemical environment on microbial thermo tolerance, particularly the beverage pH and ethanol concentration, with other chemical factors also likely to play a significant role. The present study assessed the growth and thermo tolerance of common beer and brewery related spoilage organisms in two beer styles. Once the lowest calculated PU value to provide microbial commercial sterility was determined, tunnel pasteurisation was performed and commercial sterility confirmed.

Methodology

Selection of beer

To assess the growth and pasteurisation survival post pasteurisation of the microorganisms in beer, two styles were selected: a light coloured beer and a dark beer. It has been demonstrated previously that different beer styles have an impact on bacterial growth and survival *(20)*. The light coloured beer was an ale, produced by Wychwood brewery (Witney, UK) and the dark coloured beer was a stout, produced by Meantime brewery (London, UK). To minimise the effect of alcohol concentration and pH on bacterial survival *(21)*, both beer styles selected for this study had a declared alcohol content of 4.5% ABV. The alcohol content measured by distillation was 4.6 and 4.8% ABV, for the ale and stout. The pH, measured with an AR15 pH meter (Accumet Research, USA) was 3.7 and 3.9 for the ale and stout. The bitterness measured by spectrophotometery was 22.1 and 23.5 IBU (International Bitterness Units), for the ale and stout.

Culture selection and inoculum preparation

Fourteen microorganisms were selected for this study comprising isolates that are associated with beer spoilage. A full list of the organisms is presented in Table 2.

All organisms used in this study were recovered from long-term storage (liquid nitrogen) and grown in liquid media – Wallerstein Nutrient broth (WLN; Oxoid, UK) for aerobic bacteria, and de Man, Rogosa and Sharpe (MRS; Oxoid, UK) broth for anaerobic bacteria. All cultures were grown for 5 days at 25 ± 1°C. The working stock cultures were prepared and broths with the addition of sterile glycerol (10% v/v) were stored at −70°C until required.

Prior to inoculation, all strains were adapted to the beer environment. Aliquots of 100 μL stock culture were added to 50:50 solutions of broth (WLN or MRS) and beer (ale or stout) and incubated for 5 days at 25 ± 1°C. After incubation, cells were washed by centrifugation at 3500 *g* and the resulting pellet was resuspended in either ale or stout.

Thermo tolerance in ale and stout

The thermo-tolerance of a range of common beer spoilage organisms was determined at 60°C in ale and stout. Individual strains were recovered from the working stock cultures and grown in the appropriate adaptation broth, as described above. Thereafter, cells were washed by centrifugation at 3500 *g* for 15 min. The pellets were re-suspended in 10 mL of ale or stout and the thermo resistance at 0.5 PU (60°C for 30 s) was tested using capillary tube

Table 2. Microorganisms selected for this study

Microorganism	Campden BRI code	Source
Gluconobacter oxydans	BSO395	Brewery isolate
Gluconacetobacter saccharivorans	BSO545	Brewery isolate
Acetobacter pasteurianus	BSO547	Fermented beverage
Kocuria kristinae	BSO428	Culture collection
Obesumbacterium proteus	BSO456	Isolated from vinegar
Enterobacter kobei	BSO573	Brewery isolate
Bacillus megaterium	BSO589	Beer
Lactobacillus brevis	BSO494	Beer (ale)
Lactobacillus paracasei	BSO564	Brewery isolate
Lactobacillus brevis	BSO566	Fermented beverage
Lactobacillus hilgardii	BSO600	Beer (ale)
Pediococcus cerevisiae	BSO214	Culture collection
Pediococcus pentosaceus	BSO328	Brewery isolate
Pediococcus damnosus	BSO596	Brewery isolate

method *(22)*. Briefly, 50 μL of solution containing between 10^7 and 10^8 CFU/mL was introduced into the soda glass capillary tubes G119/0,2 (Fisher Scientific, UK); the tube ends were heat sealed and processed within 15 min. As shown by Bradshaw *et al. (23)*, sealing the capillary tubes did not affect the testing solution. The sealed capillary tubes were then submerged in a water bath at the test temperatures (54, 56, 58 or 60°C) and held for a required pre-established time. Although the ramp time was not measured in this study, Jordan *et al. (18)* and Basaran-Akgul *(24)* showed that this period in glass capillary tubes was short (<10 s). The holding times quoted in this paper include the ramp-up time. The tubes were removed from the water bath, cooled in ice water, the liquid recovered in Maximum Recovery Diluent (Oxoid, UK) and the number of viable cells enumerated by spread plating. Aerobic bacteria were recovered on WLN agar after 5 days of aerobic incubation at 25 ± 1°C, anaerobic bacteria were recovered on Raka-Ray agar (RR; Oxoid, UK) following 5–7 days of anaerobic incubation at 25 ± 1°C. The log reduction (log difference of number of microorganisms before and after heat treatment [($\log_{10}$CFU/mL at T_0) – ($\log_{10}$CFU/mL at T_{End})] was then calculated and the most thermo tolerant organism determined.

Growth of bacteria in beer

All 14 microbial strains were evaluated for their ability to survive or grow in both styles of beer. Individual strains were recovered from the working stocks and grown in the appropriate adaptation broth. The cells were then washed in beer (ale or stout) by centrifugation at 3500 *g* for 15 min and the pellet re-suspended in 10 mL of ale or in 10 mL of stout. These inocula were serially diluted in Ringer's solution and 100 μL of one decimal dilution, added to 10 mL samples (ale or stout), was dispensed into 25 mL sterile universals to give an inoculum at a cell concentration of between 10^2 and 10^4 CFU/mL. The samples inoculated with aerobic bacteria were incubated aerobically with container lids slightly loose. The samples inoculated with anaerobic bacteria were incubated with lids tightly closed. All samples were incubated in a temperature controlled incubator at 25 ± 1°C for 14 days. The level of microbial cells was enumerated using the spread plate technique immediately after inoculation (D0), and after 7 (D7) and 14 (D14) days. Inoculated broth (1 mL) was serially diluted with sterile Ringer's solution and 100 μL aliquots of the dilutions were spread plated onto agar plates. WLN was used for the enumeration of aerobic bacteria following aerobic incubation for 3–5 days at 25 ± 1°C and RR agar was used for the enumeration of anaerobic bacteria following anaerobic incubation for 5–7 days at 25 ± 1°C. Following incubation, colonies were counted and the results expressed as CFU/mL and $\log_{10}$CFU/mL. In addition, the log reduction (logarithmic difference of number of viable cells on the day of inoculation and day of testing) was calculated and presented as a bar graph.

Determining *D*- and *z*-values

The three most thermo resistant microorganisms which would be able to grow in both ale and stout were selected for this experiment. They were *A. pasteurianus* (BSO547), *L. brevis* (BSO566) and *L. hilgardii* (BSO600). Their thermo resistance (*D*- and *z*-values) was determined at four temperatures – 54, 56, 58 and 60°C – using the capillary tube methodology. Each experiment was performed in triplicate. Preliminary trials were performed to select suitable holding times, to ensure an adequate $\log_{10}$ decrease in viable microorganisms. For each heat inactivation trial, the number of viable cells was enumerated at six holding times. For each trial, results were expressed as level of viable counts (CFU/mL), decimal log value, mean value and standard deviation. Inactivation curves (not shown) were drawn separately for each replicate and $D_{R1,R2,R3}$-values and $z_{R1,R2,R3}$-values for each replicate were calculated. Finally, mean *D*- and *z*-values and standard deviations were calculated (Table 3).

Validation in tunnel pasteuriser

As the thermo tolerance study conducted in ale and stout did not show any significant differences in the heat resistance of the tested strains, all three strains were used for the tunnel pasteurisation validation. Prior to validation, the lowest PU value that would result in at least a 6 log reduction was estimated. The estimation was based on the *D*- and *z*-values for the most heat resistant microorganism investigated in this study and the temperature profile during tunnel pasteurisation. The Process Lethality Calculator *(25)* was used to calculate both PU_{tot} (pasteurisation units of the process – eqn (1)) and L_{Tproc} (Lethality of the process – eqn (2)) *(6)*.

The PU_{tot} was calculated using following equation:

$$PU_{tot} = \sum_{0}^{t} L_T \times \Delta t_T \quad (1)$$

where L_T is the lethality rate.

$$L_T = 10^{(T-T_{Ref})/z}$$

is used for determined *z*-value or $L_T = 1.393^{(T\,-\,60)}$ for Del Vecchio *z*-value (6.94°C), and reference temperature T_{Ref} = 60°C. *T* is the temperature, T_{Ref} is the reference temperature, *z* is the *z*-value and Δt_T is the time at temperature *T*.

Table 3. *D*- and *z*- values for the three microorganisms tested

		D-values ± SD (min) and R^2									
Strain	Beer	54°C	R^2	56°C	R^2	58°C	R^2	60°C	R^2	z-values	R^2
Acetobacter pasteurianus	Ale	1.20 ± 0.08	0.967	0.49 ± 0.01	0.985	0.17 ± 0.02	0.984	0.09 ± 0.02	0.997	5.17 ± 0.32	0.992
(BSO547)	Stout	1.30 ± 0.07	0.918	0.35 ± 0.03	0.973	0.27 ± 0.03	0.983	0.14 ± 0.01	0.972	6.71 ± 0.14	0.914
Lactobacillus brevis	Ale	0.85 ± 0.03	0.978	0.39 ± 0.07	0.988	0.22 ± 0.02	0.976	0.20 ± 0.02	0.989	9.48 ± 0.37	0.908
(BSO566)	Stout	0.74 ± 0.07	0.987	0.36 ± 0.01	0.981	0.24 ± 0.01	0.975	0.15 ± 0.01	0.990	8.68 ± 0.39	0.984
Lactobacillus hilgardii	Ale	0.81 ± 0.03	0.979	0.42 ± 0.01	0.991	0.25 ± 0.01	0.982	0.07 ± 0.00	0.994	7.72 ± 0.43	0.996
(BSO600)	Stout	0.80 ± 0.03	0.996	0.46 ± 0.01	0.988	0.27 ± 0.01	0.994	0.08 ± 0.00	0.998	8.47 ± 0.09	0.999

$$L_{T\text{proc}} = \frac{PU_{\text{tot}}}{D_{\text{Ref}}} \quad (2)$$

where D_{Ref} is the D-value at reference temperature.

Temperature profiling was determined in a product bottle and the temperature was measured using a calibrated WiFi-TP – Temperature Data Logger (Corintech Ltd, UK). For the validation trial all three strains were mixed and used as a microbiological cocktail. The microbiological cocktail contained approximately the same number of cells from each strain. To show whether the pasteurisation process would be capable of inactivating over 6 log of bacterial cells, a high level of inoculation ($>10^6$ CFU/mL) was required. Thus bacteria were grown in larger volumes (first broth then broth + beer adaptation medium). Following the microbial cocktail preparation and the adaptation step, three 500 mL bottles of ale and three 500 mL bottles of stout were opened and inoculated with an aliquot of microbiological cocktail. Immediately after inoculation, bottles were capped and the content mixed for 2 min by inversion. The level of inoculation was then enumerated by spread plating 100 μL of the appropriate decimal dilutions onto RR agar for enumerating *Lactobacillus* spp. and onto WLN for the enumeration of *A. pasteurianus*. The bottles were re-capped, placed into the tunnel pasteuriser and the pasteurisation process started. Three pasteurisation trials were conducted: one for which a kill of less than 6 logs was expected so that a small number of bacteria should be recovered, for trials two and three all inoculated bacteria were expected to be inactivated, resulting in a high log reduction (>6 logs). Following pasteurisation, the number of viable bacteria was enumerated using two techniques. First, the samples were analysed by the spread plate technique where 100 μL of adequate dilutions were spread onto WLN and RR agar. In addition, 1 mL (2 × 0.5 mL) of undiluted sample was also spread plated (limit of enumeration <1 CFU/mL). Secondly, 10 mL of sample was filtered through a 0.45 μm filter (MF – membrane filtration) and viable cells recovered on WLN and RR agar (limit of enumeration <0.1 CFU/mL).

Results and discussion

Preliminary screen

Determining growth/survival of bacteria in ale and stout

The selected microorganisms demonstrated a broad range of growth/survival abilities in ale and stout. It was shown that all of the acetic acid bacteria included in the study, as well as *Kocuria kristinae* and two strains of *Lactobacillus* (BSO566 and BSO600) grew well in ale and stout. *Obesumbacterium proteus* (BSO456), *Enterobacter kobei* (BSO573), *Lactobacillus paracasei* (BSO564), *Pediococcus cerevisiae* (BSO214), *Pediococcus pentosaceus* (BSO328) or *Pediococcus damnosus* (BSO596) did not grow in either ale or stout. *Bacillus megaterium* (BSO589) only grew in ale and *L. brevis* (BSO494) only grew in stout (Figs 1 and 2).

Thermo tolerance in ale and stout

The results of the preliminary screen for thermo tolerance revealed a broad range of heat resistance between the different microorganisms that were heat challenged at 0.5 PU (30 s at 60°C). The three most thermo tolerant microorganisms were *A. pasteurianus* (BSO547), *O. proteus* (BSO456) and *L. paracasei* (BSO564) (Fig. 1). Log reductions of those three strains were smaller in stout than in ale (0.5–0.6 log for stout, and 1.2–1.9 log for ale).

Selection of strains for the thermo resistance study

Based on all results (thermo resistance and growth ability; Fig. 1) and the score plot (Fig. 2), the spoilage organisms *A. pasteurianus* (BSO547), *L. brevis* (BSO566) and *L. hilgardii* (BSO600) were found to be sufficiently thermo resistant to yield robust data for further experimentation.

Determining *D*- and *z*-values

A. pasteurianus (BSO547), *L. brevis* (BSO566) and *L. hilgardii* (BSO600) were chosen as the most resistant strains and their *D*- and *z*-values were determined. Each strain was grown separately in the appropriate liquid medium. Following adaptation, the heat inactivation experiments were conducted at four temperatures (54, 56, 58 and 60°C). The results indicated that at higher temperatures (60°C) *L. brevis* was the most heat resistant microorganism (D_{60} = 0.20 and 0.15 min, for ale and stout respectively). At the lowest temperature (54°C) *A. pasteurianus* was the most heat resistant microorganism (D_{54} = 2.20 and 1.30 min, for ale and stout respectively; Table 3).

Pasteurisation validation

Based on the *D*- and *z*- values from the capillary tube method, and the in-bottle temperature profile during pasteurisation, it was determined that 1 PU was sufficient to eliminate >6 logs of inoculated microorganisms during the pasteurisation process. The determined PUs (PU_{tot}) and the lethality of the process ($L_{T\text{Proc}}$ – logarithmic reduction of inoculated microorganisms achieved by the process) are shown in Table 4. The PU_{tot} and $L_{T\text{Proc}}$ values were different when using the experimentally determined *z*-values or the Del Vecchio *z*-value. Lower PU_{tot} and $L_{T\text{Proc}}$ values were determined when the *z*-value used for the calculation was lower than Del Vecchio's and higher values of PU_{tot} and $L_{T\text{Proc}}$ were determined when the *z*-value used for the calculation was higher than Del Vecchio's. For example, such a difference can be seen for *A. pasteurianus* in the ale at 52°C. Using the *z*-value determined in this study gave only a 2.3 log reduction in cell numbers for this process, whereas using Del Vecchio's *z*-value, an over-estimated 7.9 log reduction was calculated.

Three validation pasteurisation trials were performed. The first trial (T1) was conducted at 0.7 PU, the second (T2) at 1.6 PU and the third (T3) at 3.0 PU (calculated using the Del Vecchio *z*-value). For each trial, one inoculated bottle of ale, one inoculated bottle of stout and one non-inoculated bottle filled with tap water, containing the temperature probe (for temperature profiling), were placed in the middle of the pasteuriser and the trial performed. Preliminary temperature profiling trials showed that a pasteuriser setup of 45 min ramp time, 1 min holding time at 52°C and cooling to 35°C for 25 min would achieve ~0.75 PU. A 45 min ramp time, 1 min holding time at 54°C and cooling to 35°C for 25 min would achieve ~1.6 PU and a 45 min ramp time, 1 min holding time at 56°C and cooling to 35°C for 25 min would achieve ~3 PU. For each trial the temperature in the un-inoculated bottle was measured and logged using a WiFi-TP – Temperature Data Logger. The temperature profiles of the three pasteurisation runs are presented in Fig. 3. Although the required temperature was not reached, the calculated cumulative PU values were within the expected assumptions. For the first trial, the temperature reached 50.8°C and the calculated cumulative PU value was 0.7; for the second trial

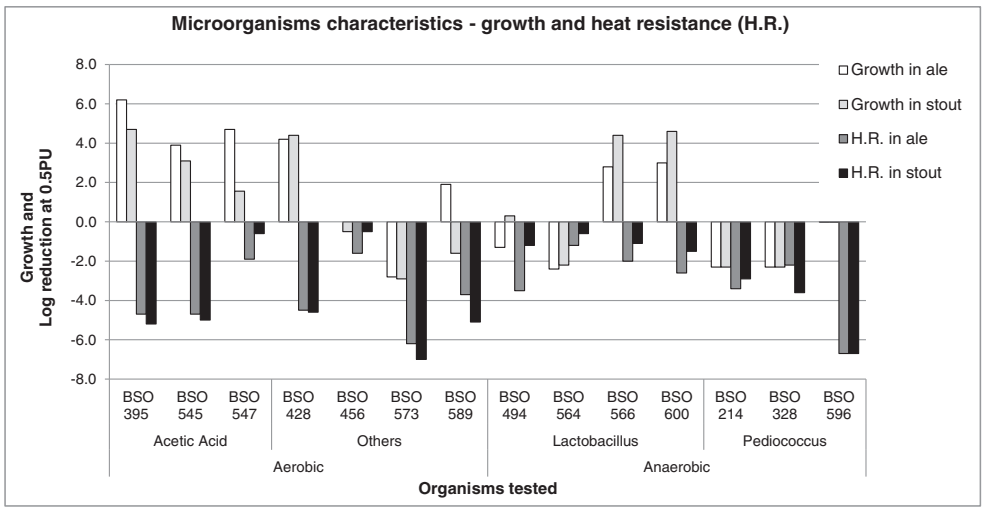

Figure 1. Microorganism characteristics – growth and heat-resistance (HR) in ale and stout.

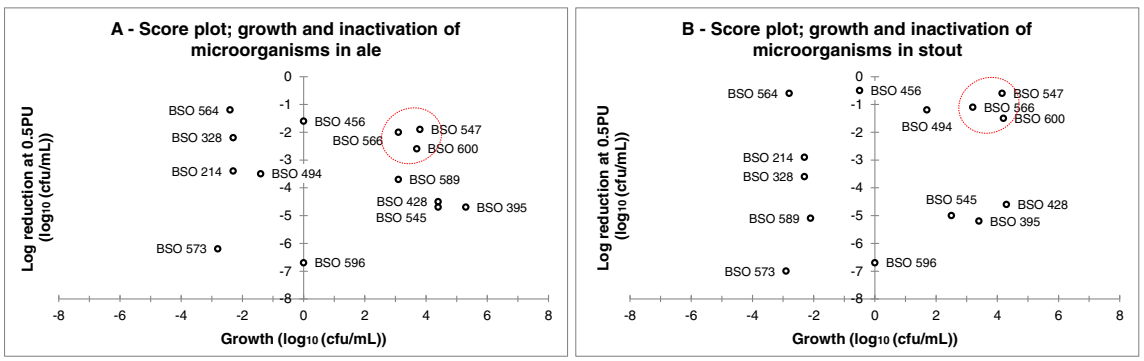

Figure 2. Score plots; growth and inactivation of microorganisms in (a) ale and (b) stout. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 4. Calculated PU_{tot} and L_{TProc} of the validation process

Microorganism	Temperature (°C)	Beer	Campden BRI trial – various *z*-values		Del Vecchio trial – z = 6.94	
			PU_{tot}	L_{TProc}	PU_{tot}	L_{TProc}
Acetobacter pasteurianus (z_{ale} = 5.17, z_{stout} = 6.71)	52	Ale	0.2	2.3	0.7	7.9
		Stout	0.6	4.5	0.7	5.1
	54	Ale	0.6	6.9	1.6	17.6
		Stout	1.4	10.2	1.6	11.3
	56	Ale	1.5	16.2	3.0	32.9
		Stout	2.8	19.6	3.0	21.2
Lactobacillus brevis (z_{ale} = 9.48, z_{stout} = 8.68)	52	Ale	2.0	10.0	0.7	3.6
		Stout	1.5	10.2	0.7	4.8
	54	Ale	3.5	17.5	1.6	7.9
		Stout	2.9	19.0	1.6	10.6
	56	Ale	5.5	27.5	3.0	14.8
		Stout	4.7	31.1	3.0	19.7
Lactobacillus hilgardii (z_{ale} = 7.72, z_{stout} = 8.47)	52	Ale	1.1	8.1	0.7	5.5
		Stout	1.4	8.9	0.7	4.5
	54	Ale	2.1	16.3	1.6	12.2
		Stout	2.7	16.8	1.6	9.9
	56	Ale	3.7	28.6	3.0	22.8
		Stout	4.5	27.9	3.0	18.5

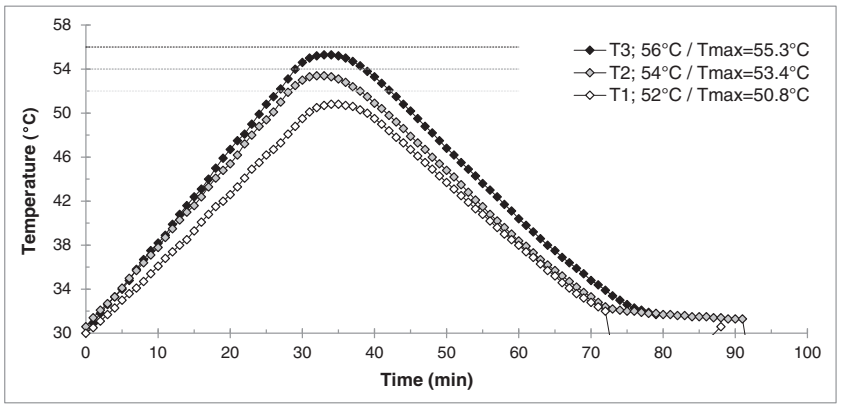

Figure 3. Temperature profiles for the three tunnel pasteurisation validation trials.

Table 5. Pasteurisation validation results

Trial no. – maximum temperature	PU	Beer	Test times	*Lactobacillus* (BSO566 and BSO600) CFU/mL	Log10 (CFU/mL)	δLog	*Acetobacter pasteurianus* (BSO547) CFU/mL	Log10 (CFU/mL)	δLog
T1, T_{max} = 50.8°C	0.7	Ale	Start (SP)	6.2×10^7	7.8	>8.8	5.0×10^7	7.7	4.8
			End (SP)	$<1.0 \times 10^0$	<0.0		8.3×10^2	2.9	
			End (MF)	$<1.0 \times 10^{-1}$	<−1.0		$>3.0 \times 10^1$	>1.5	
		Stout	Start (SP)	4.8×10^7	7.7	>8.7	5.2×10^7	7.7	7.5
			End (SP)	$<1.0 \times 10^0$	<0.0		1.0×10^0	0.0	
			End (MF)	$<1.0 \times 10^{-1}$	<−1.0		1.8×10^0	0.3	
T2, T_{max} = 53.4°C	1.6	Ale	Start (SP)	4.9×10^7	7.7	>8.7	4.7×10^7	7.7	>8.7
			End (SP)	$<1.0 \times 10^0$	<0.0		$<1.0 \times 10^0$	<0.0	
			End (MF)	$<1.0 \times 10^{-1}$	<−1.0		$<1.0 \times 10^{-1}$	<−1.0	
		Stout	Start (SP)	4.1×10^7	7.6	>8.6	5.2×10^7	7.7	>8.7
			End (SP)	$<1.0 \times 10^0$	<0.0		$<1.0 \times 10^0$	<0.0	
			End (MF)	$<1.0 \times 10^{-1}$	<−1.0		$<1.0 \times 10^{-1}$	<−1.0	
T3, T_{max} = 55.3°C	3.0	Ale	Start (SP)	4.9×10^7	7.7	>8.7	4.7×10^7	7.7	>8.7
			End (SP)	$<1.0 \times 10^0$	<0.0		$<1.0 \times 10^0$	<0.0	
			End (MF)	$<1.0 \times 10^{-1}$	<−1.0		$<1.0 \times 10^{-1}$	<−1.0	
		Stout	Start (SP)	4.1×10^7	7.6	>8.6	5.2×10^7	7.7	>8.7
			End (SP)	$<1.0 \times 10^0$	<0.0		$<1.0 \times 10^0$	<0.0	
			End (MF)	$<1.0 \times 10^{-1}$	<−1.0		$<1.0 \times 10^{-1}$	<−1.0	

SP, Spread plate; MF, membrane filtration.

the temperature reached 53.4°C and the cumulative PU value was 1.6; and for the third trial, the temperature reached 55.3°C and the cumulative PU value was 3.

The level of inoculated bacteria before and after pasteurisation was determined (Table 5). The results from Trial 1 showed that the inoculated microorganisms were not completely inactivated. Only 4.8 and 7.5 log reductions were achieved for *A. pasteurianus* in ale and stout, respectively, but the two *Lactobacillus* spp. were completely inactivated. The calculated PU for this trial (Trial 1) was 0.72. In trials 2 and 3 (T2 and T3) all inoculated microorganisms were inactivated and achieved log reductions of over 8.7 and 8.8 for ale and stout respectively.

Conclusions

Predicting the microbial stability and shelf-life of beer is challenging. Factors such as alcohol content, pH and the presence of hop compounds are known to be important in determining microbial growth and beer spoilage *(3)*. Although some guidelines exist, optimising the pasteurisation regime for different beer styles can be time consuming and can often lead to under- or over pasteurised products. This study used a laboratory based method to screen common beer spoilage organisms for their ability to grow in two beer styles and survive thermal treatment. Although the authors are aware that the long term storage of micro-organisms may indeed result in altered characteristics, all reasonable care was taken during the study to ensure that the organisms exhibited phenotypic traits associated with the genus. All organisms were screened by microscopy to ensure normal cell morphology and cultures were streaked onto nutrient agar to ensure a uniform colony morphology. Finally, before the experiments were performed, all strains were examined to ensure their ability to grow in the test beers. Together these physiological checks ensured that the organisms in this study met the basic criteria for spoilage organisms. The results demonstrated the varying abilities of microorganisms to grow in

the two beer styles. The heat inactivation trial performed at 0.5 PU showed inactivation levels ranging from 0.5 log for the most resistant microorganism to 7 log reduction for the most heat sensitive microorganism. The three most heat-resistant microorganisms able to grow in the beers were *A. pasteurianus*, *L. brevis* and *L. hilgardii*. The thermo tolerance of these bacteria in ale and stout were similar.

Based on the bacteria and beers used in this study, it was shown that the viable cell concentration in ale and stout beers was reduced to achieve 'commercial sterility' at significantly lower PU values than those recommended by the EBC *Manual of Good Practice (6)*. However, it should be borne in mind that the EBC guidelines were compiled >20 years ago and hygiene in breweries has greatly improved since then. Accordingly, it may not be so surprising that lower PUs are now sufficient to achieve stability. The EBC manual recommends using a minimum of 20 PU for ale and stout. The results from the present study indicated that a >8.7 log reduction in the cell numbers of the selected organisms was achieved at just 1.59 PU. It has to be borne in mind that this study only focused on the vegetative forms of bacteria. The most heat resistant morphological forms of bacteria and yeast are spores *(26)*, which were not investigated in this study. Further studies should focus on the heat inactivation of heat resistant yeast ascospores which are potential beer spoilers.

This study demonstrated that the *z*-values of the three most heat resistant bacteria were between 5.17 and 9.48°C and, although the *z*-value (6.94°C) reported by Del Vecchio *et al. (8)* was within this range, it was not possible to confirm or refute this value from the findings of this study. However, the calculated lethality of the validation pasteurisation process (L_{TProc}) conducted in this study was correct, and confirmed by the level of recovered microorganisms only when the *D*- and *z*-values determined in this study were used. When the Del Vecchio *z*-value was used, the calculated lethality of the process was not confirmed by the level of recovered microorganisms. Using Del Vecchio's *z*-value under- or over estimated the lethality of the process. This suggests that Del Vecchio's *z*-value was not valid for this scenario.

To the authors' knowledge, this study represents the first fully validated use of a laboratory based capillary method to determine the minimum pasteurisation regime for different beer styles based on the thermo tolerance of known beer spoilage organisms. This work has demonstrated the importance of robust laboratory scale methods to optimise the pasteurisation process in the brewery. Further studies, conducted on a broad range of organisms (including spore-forming bacteria and yeast) and other beer styles, are still required to ensure brewers are producing optimally pasteurised products for consumers.

Acknowledgments

This project was funded by the Brewers' Research & Education Fund and the support of Steve Livens, the Policy Manager, Product Assurance and Supply Chain at British Beer & Pub Association was greatly appreciated. Finally, the technical support of Belén Pérez throughout the study was greatly appreciated.

References

1. Back, W. (1994) Secondary contamination in the filling area, *Brauwelt. Int. 4*, 326–328.
2. Thelen, K., Beimfohr, C., and Snaidr, J. (2006) Evaluation study of the frequency of different beer-spoiling bacteria using the VIT analysis, *Tech. Q. Master Brew. Assoc. Am. 43*, 31–35.
3. Sakamoto, K., and Konings, W. N. (2003) Beer spoilage bacteria and hop resistance, *Int. J. Food Microbiol. 89*, 105–124.
4. Bokulich, N. A., and Bamforth, C. W. (2013) The microbiology of malting and brewing, *Microbiol. Mol. Biol. Rev. 77*, 157–172.
5. Zufall, C., and Wackerbauer, K. (2000) The biological impact of flash pasteurization over a wide temperature interval, *J. Inst. Brew. 106*, 163–168.
6. EBC Technology and Engineering Forum (1995) *Beer Pasteurisation: Manual of Good Practice*, Getränke-Fachverlag Hans Carl, Nürnberg.
7. Cao, L., Zhou, G., Guo, P., and Li, Y. (2011) Influence of pasteurizing intensity on beer flavor stability, *J. Inst. Brew. 117*, 587–592.
8. Del Vecchio, H. W., Dayharsh, C. A., and Baselt, F. C. (1951) Thermal death time studies on beer spoilage organisms, *Proc. Am. Soc. Brew. Chem. 50*, 45–50.
9. Boulton, C., and Quain, D. (2001) *Brewing Yeast and Fermentation*, Blackwell, Oxford.
10. O'Connor-Cox, E. S. C., Yiu, P. M., and Ingledew, W. M. (1991a) Pasteurization: Thermal death of microbes in brewing, *Tech. Q. Master Brew. Assoc. Am. 28*, 67–77.
11. O'Connor-Cox, E. S. C., Yiu, P. M., and Ingledew, W. M. (1991b) Pasteurization: industrial practice and evaluation, *Tech. Q. Master Brew. Assoc. Am. 28*, 99–107.
12. McCaig, L., Egan, L., Schisler, D., and Hahn, C. W. (1978) Development of required time temperature relationships for effective flash pasteurization, *J. Am. Soc. Brew. Chem. 36*, 144–149.
13. Tsang, E. W. T., and Ingledew, W. M. (1982) Studies on the heat resistance of wild yeasts and bacteria in beer, *J. Am. Soc. Brew. Chem. 40*, 1–8.
14. Molzahn, S. W., Hockney, R. C., and Kelsey, P. (1983) Factors influencing the flash pasteurisation of beer, *Proc. Eur. Brew. Conv. Congr. London, IRL Press, Oxford,, 19*, 255–262.
15. Buzrul, S. (2007) A suitable model of microbial survival curves for beer pasteurization, *LWT – Food Sci. Technol. 40*, 1330–1336.
16. Wray, E. (2015) Reducing microbial spoilage of beer using pasteurisation, in *Brewing Microbiology*, (Hill, A. Ed.), pp. 253–269, Woodhead, Cambridge.
17. Reveron, I. M., Barreiro, J. A., and Sandoval, A. J. (2005) Thermal death characteristics of *Lactobacillus paracasei* and *Aspergillus niger* in Pilsen beer, *J. Food Eng. 66*, 239–243.
18. Jordan, K. N., and Cogan, T. M. (1999) Heat resistance of *Lactobacillus* spp. isolated from Cheddar Cheese, *Lett. Appl. Microbiol. 29*, 136–140.
19. Adams, M. R., O'Brien, P. J., and Taylor, G. T. (1989) Effect of the ethanol content of beer on the heat resistance of a spoilage *Lactobacillus*, *J. Appl. Bacteriol. 66*, 491–495.
20. Vriesekoop, F., Krahl, M., Hucker, B., and Menz, G. (2012) 125th Anniversary Review: Bacteria in brewing: The good, the bad and the ugly, *J. Inst. Brew. 118*, 335–345.
21. Menz, G., Aldred, P., and Vriesekoop, F. (2011) Growth and survival of foodborne pathogens in beer, *J. Food Protect. 74*, 1670–1675.
22. Jordan, J. S., Gurtler, J. B., Marks, H. M., Jones, D. R., and Shaw, W. K. (2011) A mathematical model of inactivation kinetics for a four-strain composite of *Salmonella* Enteritidis and Oranienburg in commercial liquid egg yolk, *Food Microbiol. 28*, 67–75.
23. Bradshaw, J. G., Peeler, J. T., and Twedt, R. M. (1977) Thermal inactivation of ileal loop-reactive *Clostridium perfringens* type A strains in phosphate buffer and beef gravy, *Appl. Environ. Microbiol. 34*, 280–284.
24. Basaran-Akgul, N. (2013) Comparative study of thermal kinetics for *Clostridium sporogenes* PA 3679 inactivation using glass capillary tube and aluminum tube methods in carrot juice and phosphate buffer, *J. Pure, Appl. Microbiol. 7*, 117–124.
25. Rachon, G. (2017) Process lethality calculator, 4 December 2017. Available from: https://www.researchgate.net/publication/321497076_Process_Lethality_Calculator_PLC_-_GRachon. (accessed 26 December 2017).
26. Milani, E. A., Gardner, R. C., Filipa, V. M., and Silva, F. V. M. (2015) Thermal resistance of *Saccharomyces* yeast ascospores in beers, *Int. J. Food Microbiol. 206*, 75–80.

