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WORLD BREWING CONGRESS 2016

NEW LAB-SCALE METHOD FOR DETERMINING PASTEURISATION REQUIREMENTS IN BREWERIES USING THE HIGHLY TOLERANT SURROGATE ORGANISM *ZYGOSACCHAROMYCES BAILII*

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World Brewing Congress
August 13-17, 2016
Sheraton Downtown Denver
Denver, CO 80202, U.S.A.

Introduction

From a microbiological perspective beer is an inherently stable product and, in general, provides an inhospitable environment for most spoilage organisms. However, in recent years the alcoholic beverage sector has seen considerable growth in a range of novel drinks, which include beers and ciders mixed with various other ingredients such as fruit juices, concentrates, extracts and also a wide variety of botanicals. In addition, a greater number of zero alcohol beers and unfermented malt-based drinks are available to consumers.

Many novel drinks are lower in alcohol than standard beers or contain higher levels of sugars and, as a result, require different pasteurisation regimes to ensure product stability on-shelf. Insufficient pasteurisation increases the likelihood of a safety or quality issue, whilst excessive pasteurisation may result in off-flavour development or reduction in positive sensory attributes, and will certainly provide unwelcome higher energy costs.

At present, very little knowledge or data is available to determine the level of pasteurisation required for these types of products. This project assessed the suitability of an established laboratory based protocol, already used to assess soft drinks, to understand its potential for investigating the optimal pasteurisation regimes for alcoholic beverages.

Although not generally regarded as a beer spoilage organism, *Zygosaccharomyces bailii* was used in this investigation as a yeast capable of producing heat-resistant spores, thus representing a realistic worst-case scenario.

Methods

Organism:
Spores of *Zygosaccharomyces bailii*

Challenge tests:
100 ml of each of the four drinks was inoculated with *Z. bailii* cells to 10^2 cells per ml and incubated at 25 ^{0}C for 14 days (shaking at 250 rpm). Colony forming units were enumerated by plate counts (WLN agar) at time zero and after 14 days. The log of final cell count divided by the log of initial cell count was calculated in each case.

Test products:
Four commercially available drinks: 4% ABV beer, 2% ABV Radler, 0% ABV beer and 0% ABV malt-based drink

Small-scale pasteurisation experiments:
50 µl of inoculated de-gassed product was sealed in 100 mm length capillary tubes, immersed in a water bath at three temperatures for 20, 40, 60, 80 and 100 seconds, immediately cooled by immersion in 2 L of 5% (v/v) hydrogen peroxide and then 2 L of sterile deionised water. The capillary tubes were broken in universals containing 5 ml of sterile maximum recovery diluent. Enumeration of surviving cells was determined by plate counts (WLN agar) and there were 3 replicates for each data point.

Data:
D values (time for 1 log reduction in viable count at a given temperature) at the various temperatures were calculated for each drink as were the z values (temperature increase required for a 1 log reduction in D value) in each drink.

Results and discussion

Challenge test

The results of the challenge test established that *Z. bailii* was capable of growing in all four drinks under the test conditions (Figure 1). Growth was highest in the 4% ABV beer (3 log increase in cell count) with growth in the other three drinks much lower (1.1 to 1.6 log increase in cell count). *Z. bailii* is primarily known as a spoiler of fruit juice. However, it is resistant to high alcohol levels (≥15 % ABV), low pH (≤3.0) and low nutrient environments (Erickson and McKenna, 1999). The products contained a range of alcohol concentrations and pH (Table 1), with the growth data suggesting that *Z. bailii* showed the best growth with a pH ~4.5; the presence of 4 % ABV in the beer sample had no inherent detrimental effect on survival of *Z. bailii* under the conditions used.

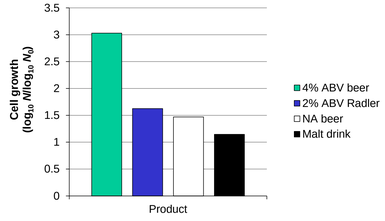

Fig. 1: Growth of *Z. bailii* in the four products after 14 days

	ABV (%)		pH	
Product	t0	14 days	t0	14 days
4 % ABV beer	4.0	4.0	4.55	4.60
2 % ABV Radler	2.0	2.0	3.10	3.17
NA Beer	<0.05	0.2	4.26	4.30
Malt drink	<0.05	<0.05	4.90	5.07

Table 1: Alcohol concentration and pH of the tested drinks before and after the challenge test

Lab-based pasteurisation protocol

The data for all four drinks at 55 ^{0}C and 60 ^{0}C was found to be very precise as can be seen from the narrow error bars in Figure 2. The data at 65 ^{0}C was less precise presumably because the decimal reduction time was short, making accurate enumeration very difficult due to low viable cell counts. Indeed, for the 4% ABV product, in order to obtain a z value the protocol had to be run at 63 ^{0}C.

Table 2 shows the D values for the spores in each drink at the various temperatures. At 55 ^{0}C the D values are very different across the data set, showing the significance of % ABV on kill rate and the clear inverse relationship. Figure 3 shows the D_{60} value versus % ABV which, in the beer based products, has a very high correlation. The malt-based beverage has a higher D_{60} value than the 0% ABV beer, which is presumably due to the protective effects of high sugar content.

The table also shows the z values for *Z. bailii* spores in the four drinks, which are reasonably consistent at around 8 ^{0}C.

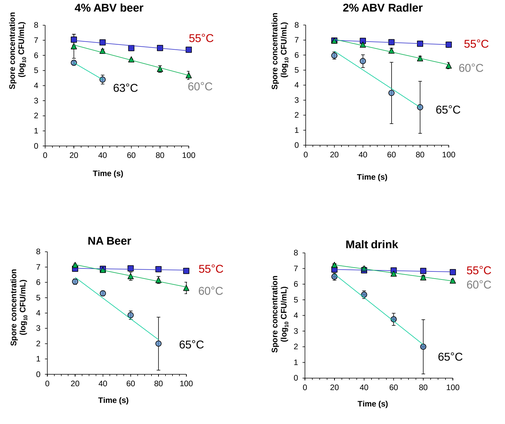

Fig. 2: Death kinetics for *Z. bailii* spores at 3 temperatures in each product

	D values (minutes)			
Temperature (°C)	4% ABV Beer	2% ABV Radler	NA Beer	NA malt drink
55	1.86	4.38	11.37	8.75
60	0.70	0.83	0.94	1.29
63	0.28			
65		0.25	0.31	0.25
z value (°C)	8.15	8.35	7.78	8.01

Table 2: D values and z values for *Z. bailii* spores in each of the products

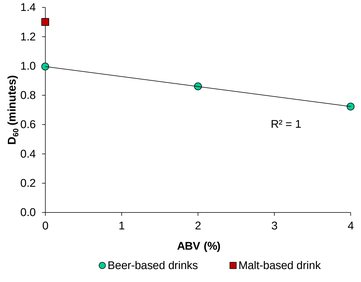

Fig. 3: Correlating % ABV of products with D_{60} values

Conclusions

The data clearly demonstrates that the lab-based pasteurisation protocol provides robust and precise data on the kill rates for beers and malt-based beverages at a range of % ABV. It is relatively quick and can be used to assess a large number of time/temperature combinations without the need for production scale trials. Additional experiments in our pilot plant facilities using a tunnel pasteuriser have validated the data from the laboratory (data not shown). This approach can be used to accurately determine the PUs required to thermally process new and existing beverages using equipment found in most microbiology laboratories. To generate the most robust data possible, the authors suggest that the laboratory-scale experiments and tunnel pasteurisation validation should be performed on a case-by-case basis for each beverage.

The z values provided by the experiments have particular significance because pasteurisation regimes in breweries are based almost entirely on a single paper published in 1951 by Del Vecchio *et al* in which a z value of 6.92 ^{0}C was postulated for beer. Our limited work here suggests that 8 ^{0}C may be a better figure to use, and could have significant implications in PUs required for product stability, but further work with a broader range of microorganisms and beers would be required to verify that hypothesis.

References

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Erickson, J.P. and McKenna, D.N. 1999. *Zygosaccharomyces*. In: Robinson, R.K., Batt, C.A., Patel, P.D. (Eds.), Encyclopedia of Food Microbiology, vol. 3. Academic Press, London, pp. 2359-2365.