High pressure processing and thermosonication of beer: comparing the energy requirements and *Saccharomyces cerevisiae* ascospores inactivation with thermal processing and modeling

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Abstract

In this research, pasteurization of beer by nonthermal high pressure processing (HPP) and thermosonication (TS) were compared with thermal pasteurization. The inactivation of *Saccharomyces cerevisiae* ascospores in beer was studied and modelled for HPP at 200, 300 and 400 MPa, and for TS at 50, 55 and 60°C with an acoustic energy density of 16.15 W/mL. The energy requirements for equivalent ascospore inactivation by HPP, TS, and thermal processes were compared. For the same processing time, ascospore inactivation was greatest with HPP, followed by 60°C TS, then 60°C thermal processing. Nonlinear survival curves which could be described by the Weibull model were observed for both HPP and TS. To achieve a 2.5 log reduction in ascospores, HPP required 77.4 kJ/L compared with 188.8 kJ/L for thermal processing and 2612.1 kJ/L for TS. HPP and thermosonication may be alternatives to thermal

beer pasteurization, achieving greater log reductions in *S. cerevisiae* ascospores with shorter processing times (TS and HPP) or less energy (HPP).

Keywords: *HPP, ultrasound, heat, spore, inactivation kinetics, energy*

1. Introduction

Beer is a beverage of low alcohol content, commonly around 4-5%. One of the last steps in the industrial production of beer is thermal pasteurization, which is performed to stabilize the beer and increase its shelf-life. ~~The global consolidation of the beer market means that~~ A prolonged shelf-life is vital but consumers are also becoming more discerning about the quality of beer as a result of the craft and specialty beer movement. Because thermal pasteurization can have negative effects on the beer's organoleptic properties, a method of pasteurization that does not affect the beer's sensory characteristics is of great interest to the brewing industry. Beer contains carbon dioxide, alcohol, and hops, all of which are natural antimicrobials, so a mild pasteurization is effective for its stabilization at room temperature (Silva & Gibbs, 2009; Silva *et al*., 2014). The pasteurization measure for beer is the pasteurization unit (PU). The minimum thermal pasteurization applied by breweries is 15 PU or 15 min at 60°C or equivalent at other temperature (Baselt, 1958), which targets the vegetative yeast grown during the fermentation of the beer.

Saccharomyces cerevisiae is a yeast used for brewing and is often the most abundant microorganism detected in the beer after fermentation and before pasteurization (Reveron *et al*.,

2012). The activity of *S. cerevisiae* can cause changes in the beer by releasing ethanol and carbon dioxide. Higher *S. cerevisiae* sporulation rates were registered when beer, barley, and malt extracts were added to the sporulation agar (Lin 1978; 1979). This suggests it is possible to find yeast ascospores during brewing, especially due to the adverse conditions created by the ethanol, hops and carbon dioxide, all antimicrobial beer components. Ascospores are more resistant to thermal processing than vegetative cells, so inactivation of the ascospores will also inactivate the vegetative cells (Milani *et al.* 2015).

High pressure processing (HPP), also known as high hydrostatic pressure processing (HHP) and thermosonication are two alternative methods of pasteurisation that have been suggested for the treatment of foodstuffs (Evelyn & Silva, 2015a; 2015b; 2015c; 2016; Evelyn *et al*., 2016; Farkas & Hoover, 2000; Hoover *et al*., 1989; Silva *et al*., 2012; Silva *et al*., 2015; Sulaiman *et al*., 2015a; 2015b), which could also have potential for use in the brewing industry (Buzrul *et al*., 2005a; 2005b; Castellari *et al*., 2000; Fischer *et al*., 2002; Gazle *et al*., 2001; Perez-Lamela *et al*., 2004). HPP is already used commercially for the treatment of fruit juices, meat and seafood but as yet, not for beer, although some studies have been carried out to determine the effect of HPP on microorganisms and flavour properties in beer (Buzrul, 2012; Silva *et al*., 2015). Sensory tests by Silva *et al.* (2015) revealed no significant difference in the overall flavour of untreated and HPP beers. Information about how microorganisms in beer respond to these treatments is needed in order to identify if they are valid techniques for industrial application and also how to optimize the industrial process. HPP subjects food products to pressures of between 100 and 800 MPa through a pressure-transmitting medium, usually distilled water. High pressure inactivates the microorganisms in several ways, including denaturing of enzymes, cell membrane damage and ribosome disintegration (Farkas & Hoover, 2000; Hoover *et al*., 1989). Yeasts and moulds are mostly very susceptible to inactivation by high pressure but can

be very resistant in ascosporic form (Evelyn & Silva, 2015b; Evelyn *et al*., 2016; Georget *et al*., 2015). HPP has been found to inactivate *S. cerevisiae* ascospores in orange and apple juice at pressures between 300 and 600 MPa (Zook *et al.*, 1999; Parish, 1998). These studies found that spore inactivation fitted a first-order kinetic model. In contrast, vegetative *S. cerevisiae* inactivation was nonlinear in wine (Mok *et al.,* 2006). We have carried out other studies to determine the HPP inactivation kinetics in non-alcoholic fruit mediums of two moulds' ascospores and bacterial spores, which were nonlinear and suitably modelled by the Weibull, log-logistic and modified Gompertz equations (Evelyn & Silva, 2015b; Evelyn *et al*., 2016; Silva *et al*., 2012; Uchida, 2015).

Power ultrasound is classified as ultrasonic waves with a frequency of between 20 and 100 kHz and a sound intensity ranging from 10 to 1000 W/cm^2 (Feng *et al*., 2008; Feng & Yang, 2011). Power ultrasound alone can be used for the inactivation of microorganisms. Thermosonication, the process of combining power ultrasound treatment with heat has been found to greatly improve the death rate of microorganisms compared with power ultrasound alone (Evelyn & Silva, 2015b; 2015c; Evelyn *et al*., 2016). Although thermosonic pasteurization still requires heat, it may reduce the time and temperature needed to achieve the same reduction in spoilage microorganisms as thermal processing alone, which would be advantageous for maintaining the beer's organoleptic properties. Ultrasound waves cause the cavitation of the liquid through which they propagate. The collapse of the bubbles caused by the ultrasound waves results in shock waves that rapidly change the pressure and temperature. This phenomenon causes the inactivation of bacteria, moulds and yeasts by damaging their cell membrane. The rapid change in pressure is the main mechanism of microbial inactivation (Condon *et al*., 2004; Feng & Yang, 2011; Piyasena *et al*. 2003). Bermúdez-Aguirre and Barbosa-Cánovas (2012) showed that *S. cerevisiae* in its vegetative state could be inactivated by thermosonication using 200 W

ultrasound at 24 kHz and 120 μm amplitude in combination with temperatures of between 40 and 60ºC. The modified Gompertz equation suited the inactivation kinetics best. No studies have been published to date modelling the inactivation kinetics of *S. cerevisiae* in its ascosporic form by thermosonication in alcoholic or non-alcoholic beverages.

The aim of this study was to describe the inactivation of ATCC 9080 *S. cerevisiae* ascospores in a lager beer (4% alcohol by volume) by a suitable model using HPP processing at varying pressures and thermosonication at varying temperatures, and compare these processes with conventional thermal processing. Ascospores were chosen as they are more resistant to temperature and pressure than yeast cells in their vegetative state and therefore represent more of a challenge to industry (Milani *et al*., 2015). This strain of *S. cerevisiae* is also known as *Saccharomyces pastorianus* or *Saccharomyces carlsbergensis*.

Therefore, the main objectives were: (i) to model the HPP inactivation of *S. cerevisiae* ascospores in beer; (ii) to model the thermosonication inactivation of *S. cerevisiae* ascospores in beer; (iii) to compare HPP, thermosonication, and conventional thermal inactivation of ascospores in beer; (iv) to compare the energy requirements for equivalent pasteurizations using different technologies.

2. Material and methods

2.1. Production of ascospores and beer inoculation

As mentioned ATCC 9080 *S. cerevisiae* strain is also known as *Saccharomyces pastorianus* or *Saccharomyces carlsbergensis*. The production of the *S. cerevisiae* ATCC 9080 ascospores

followed the method outlined by Xiao (2006) and updated by Milani *et al*. (2015) which produced *S. cerevisiae* ascospores suspended in a salt triton dithiothreitol (STD) solution to avoid spore aggregation. For the inoculation of the DB Export Gold lager (4.0% alc/vol Dominion Breweries, Auckland, New Zealand), the spore samples were centrifuged and washed with sterile water to remove the STD solution, centrifuged again and the water removed. The spores were then added to the desired amount of beer that had previously been filtered using a sterile syringe filter with a pore size of 0.2 µm (Sartorius AG, Germany) to ensure that the *S. cerevisiae* ascospores were the only microorganisms present in the sample. The initial concentration of ascospores was between 10^6 and 10^7 colony forming units per millilitre (CFU/mL).

2.2. High pressure processing

Five mL of filter-sterilized beer samples containing the yeast spore were sealed in 5×5 cm 154 µm thick pouches that had been previously sterilized (Caspak, New Zealand). The plastic film was composed of linear low density polyethylene and polyethylene therephthalate. The pouches containing the beer samples were then packed twice with the same plastic film, and the second bag was vacuum sealed, to avoid bursting during the depressurization phase of the HPP cycle. The pouches were placed inside a 2 L-700 Laboratory Food Processing System (Avure Technologies, Columbus, Ohio, USA) for varying processing times and pressures. The pressures applied were 200, 300 and 400 MPa and more samples were processed at early processing times when changes in the log reductions were higher. The system uses distilled water to pressurize the samples. The compression and decompression times, pressure, and temperature of the chamber throughout the processing were recorded. The compression times were 15, 26, and 45

seconds at 200, 300, and 400 MPa, respectively, and the decompression time was ≤8 seconds for the three HPP pressures tested. The initial temperature of the beer samples was 23˚C (pressure transmitting fluid was 24.6˚C) so that the temperature within the pressure chamber was never above 30ºC, ensuring a non-thermal HPP process. Once processed, the two pouches were immediately placed in ice water and refrigerated before enumeration of surviving *S. cerevisiae* ascospores. Two replicates for each HPP pressure-time conditions were carried out.

2.3. Thermosonication

The thermosonication experiments were conducted on the apparatus shown in Figure 1 at 50, 55, and 60˚C. A UP200S ultrasonic processor (Hielsher Ultrasound Technology Gmbh, Germany) was used to pass longitudinal mechanical vibrations with a frequency of 24 kHz, an amplitude of 125 μm, and an acoustic power density of 105 W/cm^2 through the sample via a 14 mm diameter sonotrode. A power of 161.6 W is calculated by multiplying the cross-sectional area of the sonotrode (1.539 cm^2) with the acoustic power density of the 14 mm probe (105 W/cm^2, according to the manufacturer's manual). The ultrasonic processor was set on continuous energy supply and no pulses were used. A water jacket was used to maintain the desired processing temperature inside the chamber. Before starting the TS of beer, the water bath was set to the desired temperature and circulated through the chamber prior to beer addition. This procedure minimized the temperature come-up time of the beer, which was negligible (≤5 sec). The temperature measurements were recorded in the water inlet and outlet.The chamber has a maximum volume of 15 mL but only 10 mL of beer was used for each test. Thus, the acoustic energy density supplied to the beer sample was equal to 16.15 W/mL (161.6 W/10 mL of beer).

The system was designed to be used in continuous flow mode of beer through the processing chamber. However, in order to achieve higher residence time and microbial inactivation, batch operation was used by filling the chamber with beer and closing the beer inlet and outlet valves. The apparatus was sterilized by passing a solution of disinfectant Vircon^{TM} diluted in distilled water (1% w/v) through the chamber using a pump. After this, the system was purged with sterile water to remove any remaining disinfectant solution and emptied. The beer was added to the chamber for processing by removing the ultrasonic processor and pipetting the sample into the top of the chamber. The ultrasonic processor was then replaced and the sample treated for the desired treatment time. The beer was removed from the chamber using a sterile pipette and kept refrigerated before enumeration of the surviving *S. cerevisiae* ascospores. Two repetitions of each processing time and temperature were carried out.

2.4. Enumeration of ascospores

Once the beer samples had been processed by the various pasteurization techniques, the surviving *S. cerevisiae* ascospores in each beer sample were enumerated using the serial dilution method. The diluted beer samples were streaked upon yeast extract peptone dextrose (YPD) agar medium consisting of 0.5% (w/v) yeast extract, 1.0% (w/v) peptone, 2.0% (w/v) dextrose and 2.0% (w/v) agar that had been autoclaved at 121ºC for 15 minutes. For each dilution, the two plates were incubated at 28ºC for 2 days. Then the number of colonies were counted in the dilutions with plates presenting a number of colonies between 30 and 300 and averaged for each tube dilution. The concentration of ascospores was calculated and the result was expressed in colony forming units per millilitre of beer (cfu/mL). For each pressure-time processing condition, the mean±SD of two processed beer samples was calculated and plotted in the charts.

2.5. Specific energy calculations for HPP, thermosonication, and thermal processes

The procedure of Sulaiman (2015) was used to estimate the energy requirements. Eq. 1 was used to determine the sensible heat to warm up the temperature of the beer before thermal, thermosonication and HPP processes:

$$Q = mc_p\Delta T \quad (1)$$

where Q is the heat energy needed to raise the beer temperature (J); m is the mass of the beer sample (kg); c_p is the beer specific heat capacity (4070 J/(kg.°C)); ΔT is the increase of beer temperature (°C). The beer pasteurization occurs at final stages of beer production, after the beer fermentation, and therefore the beer fermentation temperature (14°C) was considered the initial beer temperature in the calculations of sensible heat.

Equation 2 was used to estimate the compression work during the HPP pressurization (Smith *et al.*, 2005; Rodriguez-Gonzalez *et al.*, 2015):

$$W_{compression} = \frac{1}{2} \times \beta \times V \times P^2 \quad (2)$$

Where $W_{compression}$ is the compression work of incompressible fluid by high pressure (J); V is the volume of the chamber (m^3), P is the applied pressure (Pa), and β is the isothermal compressibility of the water (1/Pa).

With respect to thermosonication, first Eq. 1 was used to estimate the heat required to warm up 10 mL of beer before ultrasound processing. Next, the ultrasound power of 161.6 W (mentioned in section 2.3) was multiplied by the TS treatment processing time. Then the total energy was divided by 10 mL to obtain the specific energy in J/L.

2.6. Modelling

Table curve 2D version 5.01 software (Systat Inc., USA) was used to find an appropriate model for the HPP and thermosonication survival curves. The software calculated the parameters of models as well as performance indices. The mean square error (MSE) and adjusted coefficient of determination (adj R^2) were used to compare how well a model fitted the data. Low MSE values and values of adj R^2 close to unity indicate a good level of fit. The log survivors were non linear and among the models attempted Weibull was the most suitable (Eq. 3):

$$log \frac{N}{N_o} = -bt^n \quad (3)$$

where N is the concentration of surviving ascospores (CFU/mL) after processing time t (min). N_0 is the initial concentration of ascospores (CFU/mL); b and n are rate and shape parameters, respectively. When n=1, the model becomes the first order kinetics. A shape factor a shape factor less than 1 gives upwardly concave survival curves, while n>1 gives downwardly concave survival curves. The Weibull model, unlike first-order kinetics, does not assume that the whole microbial population have an equal time independent probability of inactivation.

3. Results and discussion

3.1. Modelling the HPP inactivation of S. cerevisiae ascospores

HPP tests were carried out at 200 MPa with processing times (holding times) up to 1 hour, 300 MPa with times up to 5 minutes and 400 MPa with times up to 30 seconds (Figure 2). This difference in the range of processing times was needed in order to model the inactivation at 200, 300 and 400 MPa and meant that the HPP pressure had a huge effect in the spore inactivation Spore reduction of ≥2.5 logs were obtained after 30 min, 27 s, and 12 s for 200, 300, and 400

MPa, respectively. Inactivation might occur during the compression phase of the HPP cycle, which could affect the initial shape of the survival curve, especially at 400 MPa. The log survivor as a function of time data collected was clearly nonlinear. The Weibull model fitted well the HPP inactivation of *S. cerevisiae* ascospores at different pressures as confirmed by adj R^2 values which ranged between 0.979 and 0.999, and MSE was between 0.010-0.030 (Table 1). The log-logistic was also attempted and showed good performance indexes, but is a more complex model characterized by 3 parameters and thus Weibull was a better option. Table 1 also displays the estimated Weibull model parameters for each HPP pressure. The nonlinear nature of the HPP survival curves for *S. cerevisiae* ascospores suggests that a resistant subpopulation of ascospores exists, which causes the nonlinearities (Fig. 2). The shape factor *n* of the Weibull model is less than 1 for all pressures, confirming the upward concavity of the survival curves (Fig. 2). This feature of the survival curve shows that sensitive members of the populations are destroyed at a relatively fast rate leaving behind resistant survivors. The *n* parameter was approximately constant (0.32-0.36) for the three pressures. Cunha *et al*. (1998) suggested *n* should indicate the kinetic pattern of the model, be constant and independent of the HPP pressure. As expected, the scale factor *b*, increased with the HPP pressure from 0.78 at 200 MPa to 4.46 at 400 MPa, meaning that higher pressure causes a more rapid inactivation of ascospores (Table 1).

No modelling studies were found for the inactivation of yeast ascospores in beer by HPP. However, the Weibull model has previously proved to be useful for fitting the survival curves of various microbial spores inactivated by HPP (Evelyn &Silva 2015a; 2015b; 2016; Evelyn et al. 2016). Mok *et al.* (2006) found a biphasic model for vegetative yeast inactivation in red wine, also suggesting two patterns of resistance to pressure. As opposed to our results with *S.*

cerevisiae ascospores in beer, Parish (1998) and Zook *et al.* (1999) found that the effect of HPP on yeast ascospore inactivation in fruit juice followed first-order kinetics.

3.2. Modelling the thermosonication inactivation of S. cerevisiae ascospores

Thermosonication experiments were carried out at 50, 55, and 60°C. Similar to HPP, the thermosonication *S. cerevisiae* ascospores survivors were strongly nonlinear (Fig. 3). A spore reduction of 2.5 log was readily achieved after 2.5 min at 60°C TS, whereas 50 and 55°C TS required more than 40 min. The nonlinear nature of the inactivation kinetics with upward concavity suggests that the *S. cerevisiae* ascospores in the beer sample had a range of resistances to treatment. Like HPP, as processing continued, the rate of inactivation decreased, suggesting some ascospores developed resistance to the ultrasonication. The resistance of microorganisms to thermosonication is analogous to microorganisms' resistance to pressure. This could be due to the dormant state of the spores.

The Weibull model presented good performance fittings ($0.95 \leq$ adj $R^2 \leq 0.988$; $0.009 \leq MSE \leq 0.055$) (Table 2) for the TS inactivation of *S. cerevisiae* ascospores in beer. Once again, the log-logistic model was suitable but a more complex model with 3 parameters and therefore Weibull was selected. Figure 3 shows the thermosonication survival curves for *S. cerevisiae* ascospores in beer at 50, 55 and 60°C fitted to the Weibull model and Table 2 presents the Weibull model parameters. Similar to HPP, the *n* value was approximately constant (0.34–0.37) and the *b* value increased with the TS temperature from 0.57 at 50°C to 1.81 at 60°C. Evelyn and Silva (2015d) also observed that the TS inactivation of *Clostridium perfringens* spores in beef slurry was not linear and described by the Weibull model. Regarding the inactivation of vegetative cells of *S. cerevisiae*, although Ciccolini *et al.* (1997) and Guerrero

et al. (2001) have reported first-order kinetic, Bermudez-Aguirre and Barbosa-Canovas (2012) observed shoulders which were modeled by modified Gompertz equation. The same authors found that a 7 log reduction of *S. cerevisiae* was achieved after 10 minutes at 60°C-TS with similar ultrasound conditions (24 kHz, 400 W, 120 μm), whereas in our study only a 4 log reduction was registered, confirming the higher resistance of *S. cerevisiae* in its ascosporic form.

3.3. Comparison of HPP, thermosonication, and thermal inactivation of S. cerevisiae ascospores

Figure 4 compares the first-order 60°C thermal inactivation of ATCC 9080 strain of *S. cerevisiae* ascospores taken from Milani *et al*. (2015), the same strain used in this study, 60°C thermosonication and nonthermal HPP processing at 300 MPa. A 2.5 log reduction of ascospores was achieved after 15 min, 2.5 min, and 27 sec processing of beer by thermal, TS, and HPP, respectively. Although no heating was used for HPP, a lower treatment time was required for the same log reduction of ascospores, which demonstrates that this technology is highly efficient for yeast spore inactivation and beer pasteurization. Referring to TS, although the heating of beer may cause negative effects on the beer quality, the ultrasound process can offer a reduction in the processing time from 15 to 2.5 min to achieve the same inactivation of *S. cerevisiae* ascospores compared with thermal processing alone. For example, a 1.8 log reduction of ascospores in beer was obtained after only 1 min of thermosonication. In contrast, for thermal processing of beer, approximately 10 min were needed for the same log reduction. This reduction in processing time with thermosonication may offer potential advantages in the brewing industry and productivity gains.

3.4. Specific energy requirements for equivalent pasteurization processes

Based on the minimum pasteurization of 15 PU or 15 min at 60°C established for commercial thermal processes, Milani *et al* (2015) found that this achieved 2.5 log reductions in ATCC 9080 ascospores, the strain used in the current study. The following 15 PU pasteurization processes were selected for comparison in terms of specific energy requirements: thermal processing at 60°C for 15 min, HPP at 300 MPa for 27.0 sec, and thermosonication at 60°C for 2.5 min. For the thermal and TS processes at 60°C, 188.8 kJ/L were required to heat up the beer to 60°C (Eq. 1). Then for TS the ultrasound power of 161.6 W (mentioned in Section 2.3) was multiplied by the processing time of 150 sec to give 24233 J, and divided by 10 mL, the volume of beer processed to give 2423.3 kJ/L. The final specific energy result for TS adds to 2612 kJ/L. With respect to HPP, first Eq. 1 was used to calculate a sensible heat of 36.9 kJ/L to raise the temperature to 23°C (the initial temperature of beer before HPP cycle), and then a compression work of 40.5 kJ/L during the HPP pressurization was calculated with Eq. 2 ($\beta_{32.9°C} \sim 4.5x10^{-10}$ 1/Pa), giving a total of 77 kJ/L. The results indicate that lower energy is required for HPP (77 kJ/L) than thermal processing (189 kJ/L). The difference in the energy is much higher when comparing both processes to TS process (2612 kJ/L). Moreover, to achieve 4 log reductions a 10 min 60°C-TS process required much more energy (9885 kJ/L) than the energy estimated for a 5.5 log reduction by HPP (300 MPa-5 min, 102.63 kJ/L). Most of the HPP energy is compression work and not much energy was spent to maintain the high pressure for a longer holding time. No other study has been carried out using yeast ascospore inactivation as the basis for comparing the energy requirements of different technologies. Sulaiman (2015) also estimated much higher energy needs for 15 min ultrasound at 33°C (1233 kJ/kg), compared to 65°C-thermal for 15 min (291 kJ/kg) and HPP at 600 MPa-48°C for 15 min (240 kJ/kg) of strawberry puree, all processes resulting in the same polyphenoloxidase inactivation. Regarding

apple juice processed by HPP, Jordan *et al.* (2001) estimated 483 kJ/kg for HPP processing at 500 MPa-42°C for 300 s and Bayındırlı *et al*. (2006) determined 338 kJ/kg for HPP at 350 MPa-40°C for 300 s. Sampedro *et al*. (2014) compared the energy consumption for pasteurization of orange juice by thermal (85°C-5s) and HPP (550 MPa-90s) processes using commercial size units and estimated higher energy consumption for thermal processing (38.1×10^3 kWh/year) in comparison to HPP (1.02×10^6 kWh/year).

4. Conclusions

The HPP and thermosonication processes generated accentuated nonlinear survival curves for *S. cerevisiae* ascospore inactivation in beer, which fitted a Weibull model. Both HPP and thermosonication are capable of achieving greater inactivation of *S. cerevisiae* ascospores in a shorter amount of time than traditional thermal processing, making them techniques that the brewing industry can consider as alternatives to thermal treatment. However, HPP processing appears to offer several potential advantages if implemented in the beer industry. First, HPP uses no heat during processing, which is likely to preserve the organoleptic properties of the beer. Moreover, nonthermal HPP requires less energy to achieve 15 PU in a shorter time, compared with TS and thermal processing at 60°C. This study can help industry and other researchers to design HPP and thermosonication processes for a targeted reduction in *S. cerevisiae* ascospores.

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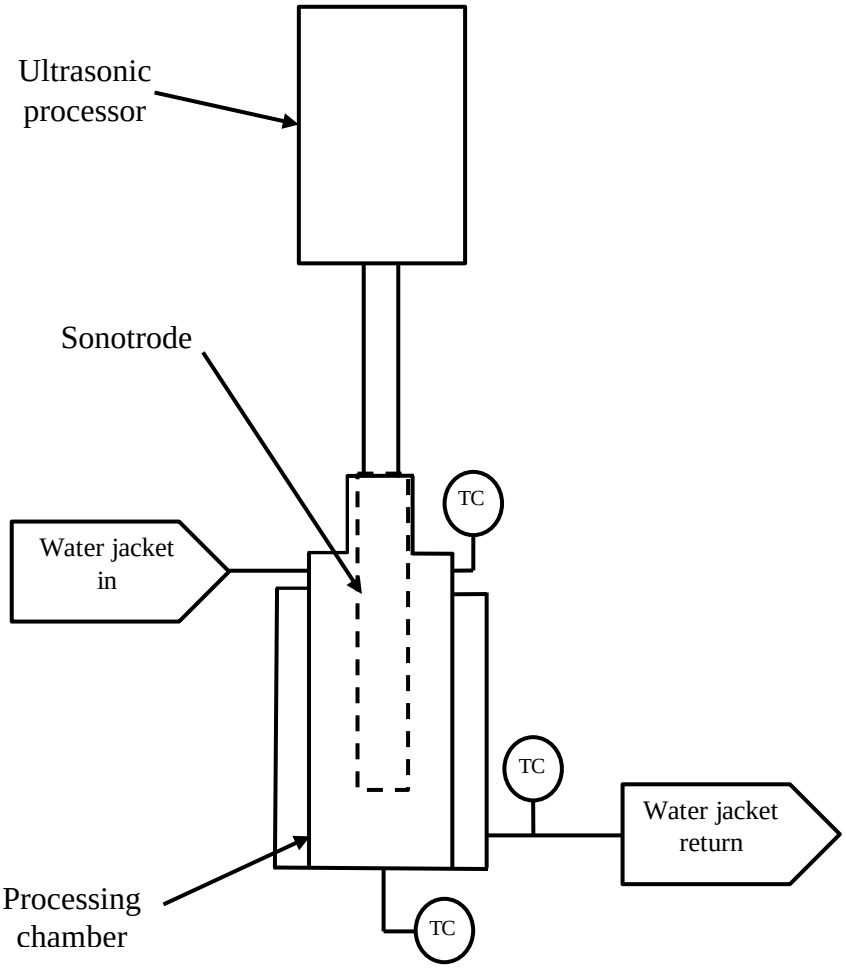

Fig. 1. Schematic diagram of the power ultrasound machine set up at the University of Auckland. TC refers to the thermocouples mounted on the machine.

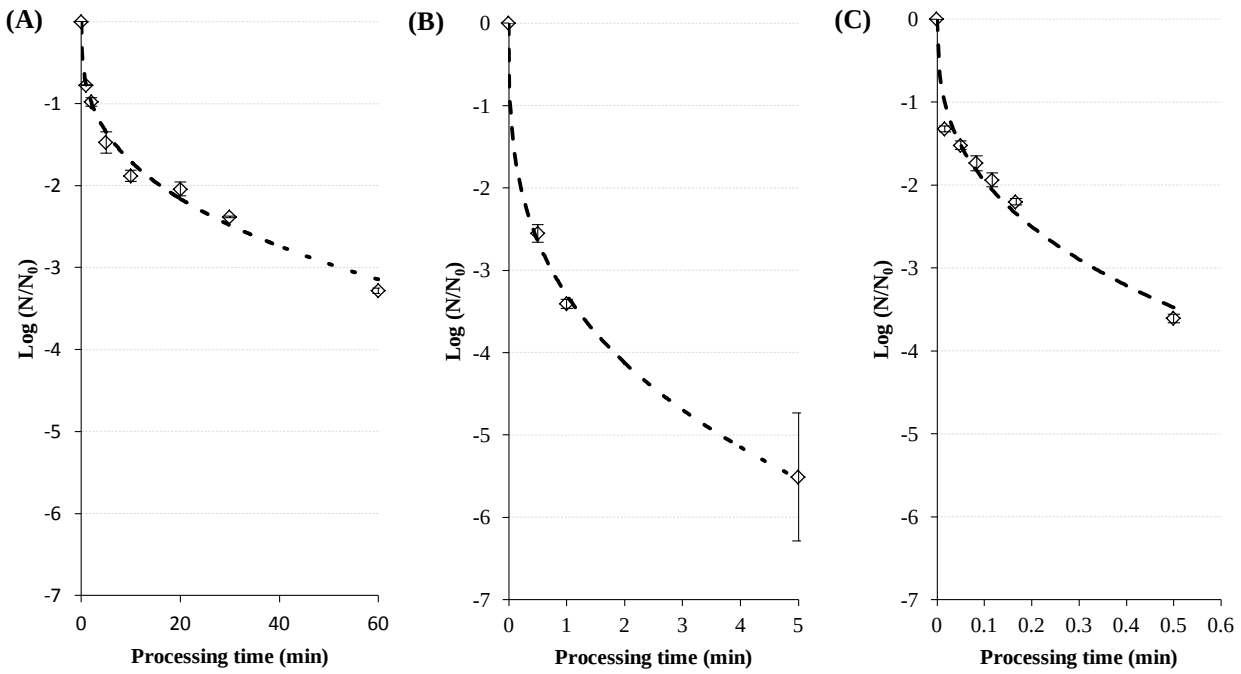

Fig. 2. Weibull model fitted to *S. cerevisiae* ascospores survivors in beer after HPP processing at (A) 200 MPa, (B) 300 MPa and (C) 400 MPa (values are average of two processed samples and error bars are standard deviation).

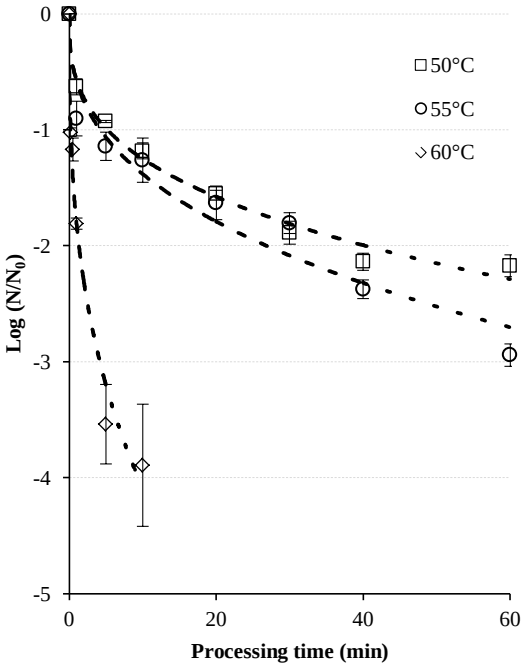

Fig. 3. Weibull model fitted to *S. cerevisiae* ascospores survivors in beer after thermosonication at 16.16 W/mL (values are average of two processed samples and error bars are standard deviation).

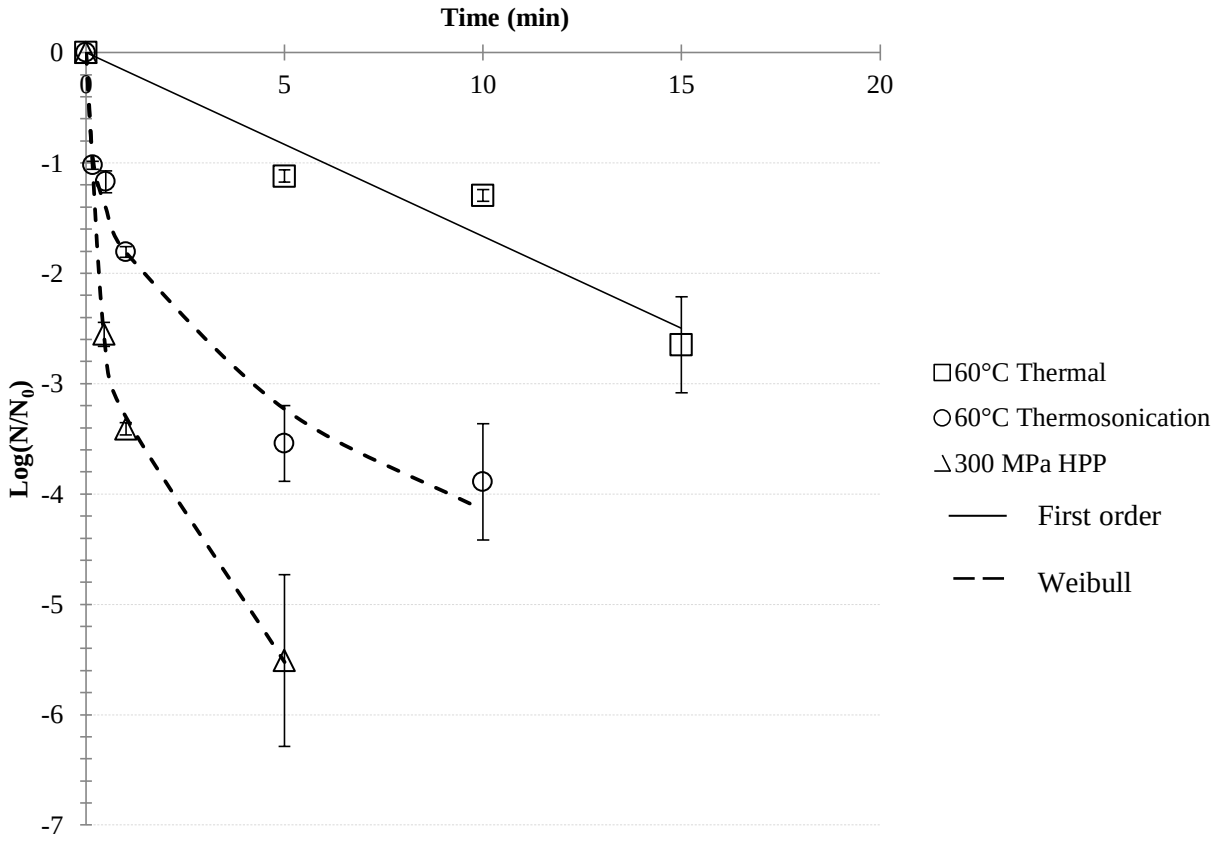

Fig. 4. Nonthermal HPP at 300 MPa and 60°C-thermosonication (16.16 W/mL) compared with 60°C-thermal inactivation of *S. cerevisiae* ascospores in beer (thermal line data were taken from Milani *et al.* 2015; values are average of two processed samples and error bars are standard deviation).

Table 1. Performance and parameters of Weibull model used to describe HPP inactivation of *S. cerevisiae* ascospores in beer.*

Pressure (MPa)	*b*±SE	*n*±SE	*Adj* R^2	MSE
200	0.78±0.06	0.34±0.02	0.999	0.013
300	3.30±0.07	0.32±0.02	0.999	0.010
400	4.46±0.09	0.36±0.03	0.975	0.030

*Adj R^2 and MSE are the adjusted coefficient of determination and Mean Square Error, respectively; in addition, the residual plots were random. *b* and *n* are the scale and shape factors from the Weibull model (Equation 3), respectively.

Table 2. Performance and parameters of Weibull model used to describe thermosonication (16.16 W/mL) inactivation of *S. cerevisiae* ascospores in beer.*

TS temperature (°C)	*b*±SE	*n*±SE	*Adj* R^2	MSE
50	0.57±0.05	0.34±0.03	0.986	0.009
55	0.58±0.12	0.37±0.06	0.942	0.048
60	1.81±0.04	0.36±0.04	0.976	0.055

*Adj R^2 and MSE are the adjusted coefficient of determination and Mean Square Error, respectively; in addition, the residual plots were random. *b* and *n* are the scale and shape factors from the Weibull model (Equation 3), respectively.