Thermal Resistance of *Saccharomyces cerevisiae* in Pilsen Beer

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ABSTRACT

J. Inst. Brew. 109(2), 120–122, 2003

The thermal resistance of *S. cerevisiae* (CMOJ896) was determined in Pilsen beer (pH = 4.28 ± 0.05; extract of original wort (EOW %) = 11.30 ± 0.08; percentage of alcohol by volume (% at 20°C) = 4.97 ± 0.05; total nitrogen content (mg/L) = 590 ± 37; bitterness units (BU) = 20.5 ± 1.3; carbonation of the beer (Vol. CO_2) = 2.89 ± 0.09; color (SRCM) = 3.3 ± 0.4). The flask method was used for an initial population of 1×10^4 cells/mL. Decimal reduction times of $D_{47°C}$ = 3.16 min, $D_{48°C}$ = 2.65 min, $D_{49°C}$ = 1.74 min and $D_{50°C}$ = 0.68 min were obtained at the temperatures studied. Values of $D_{60°C}$ = 0.01 min and z = 4.6° were obtained for this microorganism.

Key words: Beer, *Saccharomyces cerevisiae,* thermal resistance.

INTRODUCTION

Saccharomyces cerevisiae, the yeast most frequently used for the manufacture of beer, is the primary microorganism present in the converted wort after fermentation and filtration, and prior to pasteurization. Most beers are pasteurized after filling to achieve microbiological stability and to inactivate molds and yeasts that might otherwise alter and deteriorate the product after processing. A general pasteurization process of 5–15 PU (Pasteurization units, defined as one minute at 60°C) has been suggested for beer[4,12].

Information concerning the thermal resistance of *S. cerevisiae* in foods is scarce. Tsang and Ingledew[11], who studied the heat resistance of yeasts and bacteria in beer, found a very low heat resistance for yeasts. Cerny[1] reported the effect of pH on heat resistance of *S. cerevisiae,* determining a large dependence of D values on pH at 50°C, decreasing as temperature increased up to 65°C when dependence was practically nil. Hasselbeck *et al.*[3] presented a value of $D_{75°C}$ = 0.004 seconds for *S. cerevisiae* in orange juice. Shimoda *et al.*[9] found first order kinetics for the thermal resistance of *S. cerevisiae* ATCC 18824 in acidic beverages at low temperature (30 to 38°C), while studying the effect of temperature and CO_2 pressure (4 to 10 MPa). The combined effect of temperature (45–55°C) and ultrasound (20 KHz) in the heat resistance of *S. cerevisiae* in foods was studied by Lopez-Malo *et al.*[5]. The authors reported that ultrasound was capable of reducing the heat resistance of this microorganism.

No published reports were found with regards to the thermal resistance of *S. cerevisiae* in Pilsen beer. Although the thermal process applied in beer pasteurization is a low temperature process, it is desirable to apply the minimal thermal process in order to reduce undesirable organoleptic changes in the final product. Knowledge of thermal resistance parameters of the target microorganism is essential for designing the minimal thermal process required for proper pasteurization. The objective of this work was to determine the thermal resistance parameters D and z for *S. cerevisiae* in Pilsen beer.

Definitions. D value is a measure of the heat resistance of a microorganism. It is the time in minutes at a given temperature required to destroy 1 log cycle (90%) of the target microorganism. Z value reflects the temperature dependence of the reaction and is defined as the temperature change required to change the D value by a factor of 10.

MATERIALS AND METHODS

Product characterization

Thermal resistance studies were carried out in non-pasteurized Pilsen beer obtained from an industrial process. Physicochemical characteristics of non-pasteurized Pilsen beer that could affect thermal resistance were determined according to the methods presented in COVENIN[2]. The following analyses were carried out on the experimental beer: pH, extract of original wort, percent alcohol, total nitrogen content, bitterness units, carbon dioxide and colour. These parameters were determined as follows: a) pH with a Corning M-145 pH-meter; b) Extract of original wort by determining the specific gravity (20/20°C) using a beer analyser Anton Paar (Austria), previously calibrated with distilled water, to calculate the apparent and real extract to establish the original extract; c) Percent alcohol by volume: using tables of specific gravity and density at 20°C; d) Total nitrogen: by the Kjeldahl method using a Buchi-435 Digestion Unit and a Buchi-339 Distillation Apparatus (Buchi Switzerland); e) Bitterness units: using a Hewlett Packard 8452-A spectrophotometer to determine the optical density at 275 nm; f) Colour: with the same equipment at 430 nm and expressed in SRCM (standard reference color method) degrees; g) Carbon dioxide content: using a Haffmans Inpack 2000 CO_2 Meter (The Netherlands).

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Publication no. G-2003-0609-134

Preparation of the inoculum

The strain used in this work was isolated and identified from non-pasteurized Pilsen beer obtained from an industrial process[7]. The strain was identified as *Saccharomyces cerevisiae* (CMOJ896) using the current taxonomy. Under the older naming terminology this strain is designated as a lager yeast (*Sacch. uvarum* (*carlsbergensis*)) since it is able to ferment the sugar melibiose and is not able to grow at 37 C, these two tests being used in the brewing world to distinguish ale and lager strains.

Three to four loops of single colony isolates, precultured on wort agar pH 5.0 (Merck 5448) incubated at room temperature (20–23°C) for 72 hours, were transferred to 150 mL of wort broth pH 5.0 (Merck 5449) and well mixed with a magnetic stirrer at room temperature (20–23°C) for 72 hours until the apparent extract reached a value of 3 percent. The inoculum for the thermal resistance study was prepared taking 10 mL of the above prepared culture, separating the cells by centrifugation (2500 rpm for 10 min) and washing them three times with phosphate buffered saline (Sigma P-4417) 0.01 M (pH 7.4). The cell concentration was standardized at 1.0×10^6 live cells/mL by direct count in triplicate in a Neubauer chamber, using Trypan Blue to identify dead cells[7].

An inoculum with a concentration of 1.0×10^4 cells/mL of the microorganism, was established based on the concentration of this microorganism in non-pasteurized beer. The concentration of this microorganism in the non-pasteurized beer was determined previously, using Wallerstein agar (WLNM Difco N° 0424) at pH 5.5, incubated under anaerobic conditions for 5 days at 25°C. The established inoculum was approximately three logarithmic orders above the average count found in non-pasteurized beer, in order to guarantee that extreme contamination levels that could be found in non-pasteurized beer were included. The inoculum was prepared by dilution of the standardized solution prepared previously.

Preparation of the sample

Commercial pasteurized Pilsen beer was used for the thermal resistance study. The beer was filter sterilized to avoid the physiological changes that can be induced by heating that might affect the thermal resistance of the microorganism studied. The sample was sterilized by consecutive filtration with cellulose acetate membranes with pore size of 0.70, 0.45 and 0.20 μm. A sample of 25 L was prepared to have sufficient sample to avoid variability introduced by the sample composition (pH, alcohol content, extract and total nitrogen, among others) that could affect thermal resistance. The sterilized beer was kept in an amber flask at 4°C until required and tested to ensure sterility before use (by total microbial count and acid-tolerant microorganisms).

Thermal resistance study

Thermal resistance was determined by the Flask method[10] using a three-neck Woulff flask of 500 mL. A high precision thermometer (Thermo-Schneider, ± 0.1°C, previously calibrated and certified) was inserted through a neoprene septum in one neck in order to measure the temperature in the flask center. A mechanical stirrer with a long shaft, connected to a motor provided with a speed regulator, was inserted through the central neck to continuously stir and homogenize the inoculum in the medium. The third neck was used to inoculate the sterile beer, at the beginning of the experiment, and to withdraw samples during the test. This neck was covered with a cotton plug to maintain aseptic conditions during the experiment. All the glass material used, the flask, agitator and the neoprene septum were sterilized in an autoclave at 121°C for 20 min previous to commencing the experiments.

A volume of 495 mL of sterile beer was aseptically poured into the sterile Woulff flask. The flask was assembled and introduced in a heated water bath (± 0.1°C) provided with water circulation (Lauda RM6-Brinkmann) and heated to the experimental temperature. Four temperatures (47.0, 48.0, 49.0 and 50.0°C) were used in different experiments. Once the temperature was stabilized at the desired level, 5 ml of cell suspension was aseptically inoculated into the flask using the third neck in order to obtain a final cell concentration of 1×10^4 cells/mL. Samples of 1000 μL were aseptically drawn through the third neck with sterile micropipets (Gilson P-1000) every 15 seconds for a total experiment time of 6 min. Subsequently, the sample was poured in vials (1.5 mL) and immediately placed in an ice bath (1–2°C) to quench the thermal treatment. Time was measured with a certified chronometer (Supelco, appreciation: 1 s). Zero time was established after pouring the initial inoculum. The surviving population at each sampling time was determined in duplicate by plate counts using Wallerstein agar (pH 5.5) (WLM Difco 0424) with aerobic incubation for 5 days at 25°C.

Determination of thermal resistance

The survival population at each time was averaged and the thermal resistance of the strain at each temperature was determined by plotting the thermal survivor and the phantom thermal death time curves. D and z values for each strain were determined according to the literature[10]. Statistical analysis and fitting of the data were carried out with the statistical package Statgraphics.

RESULTS AND DISCUSSION

The results for the physicochemical characteristics of the medium that could affect the thermal resistance of the microorganism considered in this work were indicative of the following values (± standard deviation for 30 samples): pH = 4.28 ± 0.05; extract of original wort (EOW %) = 11.30 ± 0.08; percentage of alcohol by volume (% at 20°C) = 4.97 ± 0.05; total nitrogen content (mg/L) = 590 ± 37; bitterness units (BU) = 20.5 ± 1.3; carbonation of the beer (Vol. CO_2) = 2.89 ± 0.09; color (SRCM) = 3.3 ± 0.4.

The thermal survivor curves for the temperatures considered in this study indicated that thermal destruction followed the classical decimal reduction pattern corresponding to a first order kinetics. The data obtained are presented in Fig. 1 and adjusted using a linear regression program. Values of $D_{47°C}$ = 3.16 min, $D_{48°C}$ = 2.65 min, $D_{49°C}$ = 1.74 min and $D_{50°C}$ = 0.68 min, were obtained for the strain *S. cerevisiae* (CMOJ896) used in this study. In all cases, high correlation coefficients were obtained in the adjustments ($0.97 < r < 0.99$).

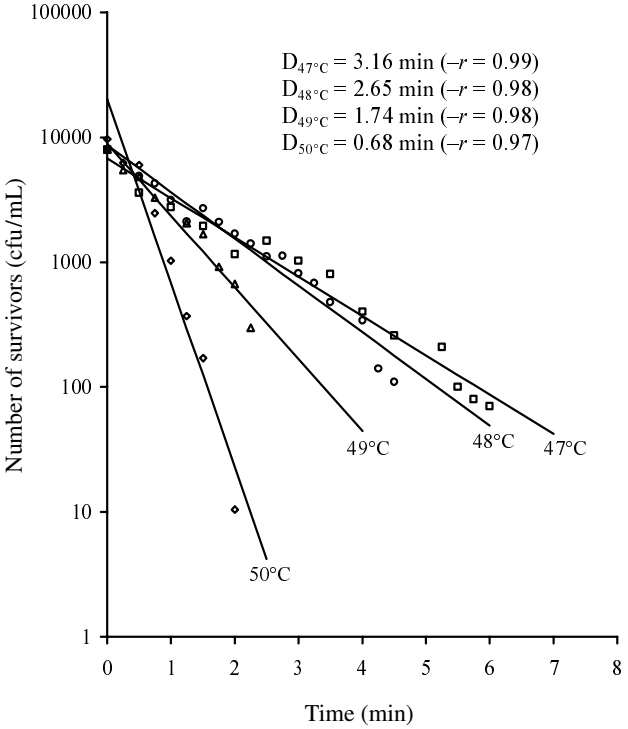

Fig. 1. Survivor curves for *S. cerevisiae* (CMOJ896) in Pilsen beer.

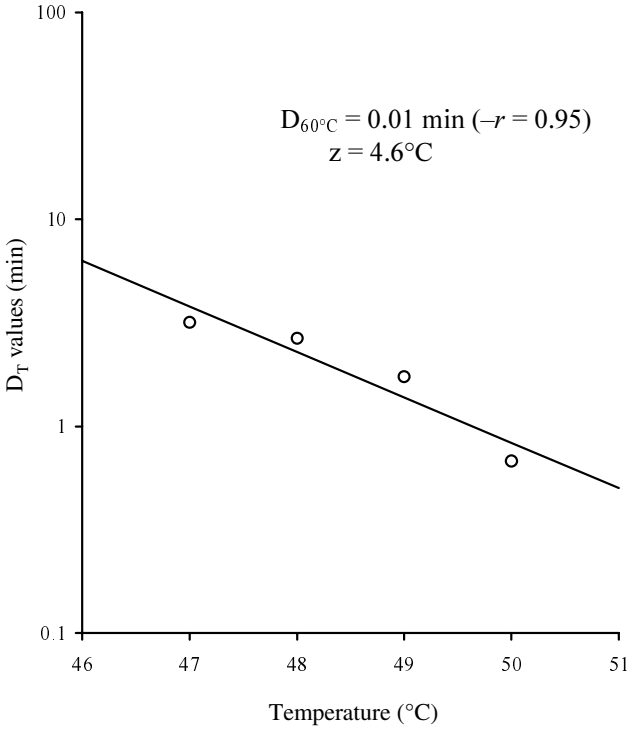

Fig. 2. Phantom thermal death time curve for *S. cerevisiae* (CMOJ896) in Pilsen beer.

The phantom thermal death curve for the above values are shown in Fig. 2. Values (at the reference temperature of 60°C) of $D_{60°C}$ = 0.01 min and z = 4.6°C were obtained by linear regression (–*r* = 0.95) for *S. cerevisiae* (CMOJ896).

Thermal resistance of yeast showed typical $D_{49°C}$ < 2 min[8]. Values of thermal resistance calculated by Put and De Jong[6] for *S. cerevisiae* isolated from soft drinks and fruit products showed values of $D_{60°C}$ = 0.11–0.32 min. For *S. cerevisiae* var. *uvarum,* typically used in the manufacture of low fermented beer, a value of $D_{60°C}$ = 0.07 min was reported. Other authors[11] studied the heat resistance of wild yeast in beer, obtaining values of $D_{49°C}$ = 1.1 min for *S. cerevisiae* var. *carlsbergensis* and $D_{53°C}$ = 0.39 min for *S. cerevisiae* var. *Willianus.*

The thermal resistance of *S. cerevisiae* (CMOJ896) isolated from Pilsen beer produced in an industrial process using the flask method gave the following results. Decimal reduction times of $D_{47°C}$ = 3.16 min, $D_{48°C}$ = 2.65 min, $D_{49°C}$ = 1.74 min and $D_{50°C}$ = 0.68 min at the temperatures studied. Values of $D_{60°C}$ = 0.01 min and z = 4.6°C were obtained for this microorganism when initial cell concentration used in the thermal resistance studies were 1×10^4 cells/mL.

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