Heat, high hydrostatic pressure (HHP) and high pulsed electric field (PEF) inactivations of *Zygosaccharomyces bailii* ascospores and vegetative cells suspended in apple, orange, pineapple, cranberry and grape juices were investigated. The ascospores exhibited a heat resistance that was more than 5–8 times greater than the vegetative cells. After 5 min of pressurization at 300 MPa, the population of vegetative cells decreased almost 5 log cycles, while the population of ascospores decreased between 0.5–1 log cycles. In each fruit juice studied, two pulses of 32–36.5 kV/cm decreased the population of vegetative cells or ascospores 3.5 to 5 log cycles.

Key words: *Zygosaccharomyces bailii*, ascospores, hydrostatic pressure, pulsed electric fields

**INTRODUCTION**

The use of high hydrostatic pressure (HHP) and high voltage electric pulses (PEF) as alternatives to thermal processing are being intensely studied (Cheftel, 1995; Qin et al., 1995a; Vega-Mercado et al., 1997). Both techniques have been highly effective in inactivating vegetative cells of bacteria, yeasts, and molds (Patterson et al., 1995; Butz et al., 1996; Palou et al., 1997).

HHP involves a nonthermal process in which foods are subjected to pressures in the range of 100–700 MPa at or around room temperature (23°C). This technology can enable increasing the shelf-life of foods and has potential applications in food texturization because the high pressures applied may denature proteins and polysaccharides (Mertens, 1995). The PEF pasteurization process involves the application of short pulses (microseconds) of high electric fields to foods placed between two electrodes. Usually the treatment is conducted at ambient or refrigerated temperatures and thus heating of the food is minimized (Qin et al., 1995b).

Fruit juices are acidic foods generally with pH<4.5. Their low pH enables the growth of yeasts, molds, and fungi, and ascospores of Z. bailii is noted for its exceptional tolerance to high concentrations of salts or sugars, acidic conditions and preservatives (Thomas and Davenport, 1985). This yeast also has the ability to form ascospores, which arise as a result of meiosis so that a typical diploid cell becomes transformed into an ascus containing haploid ascospores. The ascospore protoplast has a structure similar to vegetative cells, but the ascospore wall consists of an outer and inner coat. Yeast ascospores are more resistant to physical and chemical agents than vegetative cells (Fowell, 1974). In general, ascospores are more resistant than vegetative cells (Put and DeJong, 1982a, 1982b; Splittooste et al., 1986), ethanol and cell wall lytic enzymes (Fowell, 1975), and HHP (Previdi et al., 1994). Although the heat resistance of *Z. bailii* ascospores (Jermini and Schmidt-Lorenz, 1987) is known, no data have been published about their HHP and PEF resistance.

The objective of this work was to compare the resistance to heat, HHP, and PEF treatments of *Z. bailii* ascospores and vegetative cells suspended in apple, orange, pineapple, cranberry and grape juices.

**MATERIAL & METHODS**

**Culture preparation**

*Z. bailii* ATCC 36947 was obtained from the American Type Culture Collection (ATCC; Rockville, MD). Four 1000 mL Erlenmeyer flasks containing 450 mL of sterile yeast malt broth (YMB; Difco Laboratories, Detroit, MI) were inoculated with 50 mL of a 12h broth culture at YMB at 27°C. The yeast suspension were placed in an ice-bath for 15 min and after cooling, the yeasts were washed three times by centrifugation and resuspended in sterile YMB. After the last centrifugation the pellet was resuspended in a sterile YMB containing 10% glycerol, and a 5 mL suspension aliquot was dispensed in 15 mL vials. The vials were then stored at -20°C. The final concentration of yeast in the vials was 2 × 10^8 CFU/mL. The vegetative cells of *Z. bailii* exhibited no loss of viability or resistance to heat, HHP, or PEF during storage.

**Menstrua of treatment**

Pasteurized apple, orange, pineapple, cranberry, and grape juices were obtained from a local supermarket. No microorganisms were detected in the fruit juices before inoculation. Their pH (Table 1) and electric conductivity (Table 2) at room temperature were measured using a pH meter (Model 420A, Orion Laboratory Products Division Inc., Boston, MA) and conductivity meter (Hydac, Cambridge Scientific Instruments, Cambridge, MA).

**Heat, HHP and PEF treatments**

Heat resistance was evaluated by intro-
were inoculated to a concentration of $1 \times 10^6$ CFU/mL. Spore suspensions (5 mL) were serially diluted in sterile 0.1% peptone (Difco) water and surface-plated onto a PDA (Difco) medium. The plates were then incubated at 25°C for 5 days. The $D$ value was estimated from the slope of the regression line obtained from the linear portion of the survival curve of the plot log CFU/mL vs heating time.

**RESULTS & DISCUSSION**

**D VALUES AT 50°C OF VEGETATIVE CELLS and ascospores of Z. bailii in different fruit juices were compared (Table 1). Results showed that ascospores were more heat resistant than vegetative cells and the type of fruit juice influenced the heat resistance of both vegetative cells and ascospores. Ascospores exhibited a heat resistance more than 5–8 times greater than that of the vegetative cells. Although some investigators have reported Z. bailii and *Saccharomyces cerevisiae* ascospores over 100 fold more resistant than vegetative cells of the same strain (Put and DeJong, 1982b; Splittstoesser et al., 1986), others have reported smaller differences (5–10 fold) (Jermini and Schmidt-Lorenz, 1987; Corry, 1976).

Menstruum of treatment is an important factor that affects microbial heat resistance (Tomlins and Ordal, 1976). The vegetative and ascospore heat resistance in this study depended on the fruit juice medium. The order of effectiveness for both ascospore and vegetative cell heat inactivation was orange > cranberry > apple > grape > pineapple. The similar pH of the juices and the small reported influence of pH on the heat resistance of yeast (Beuchat, 1981; Splittstoesser et al., 1975) seemed to indicate that these differences in heat resistance were related to the fruit juice composition.

The survival curves of vegetative cells and ascospores of Z. bailii after HHP at 300 MPa were compared (Fig. 1) in different fruit juices. After 5 min of treatment the population of vegetative cells decreased almost 5 log cycles but the population of ascospores decreased between 0.5–1 log cycles. The HHP resistance of vegetative cells in fruit juices was

<table>
<thead>
<tr>
<th>Juice</th>
<th>Electric conductivity (µS/cm)</th>
<th>Peak voltage (kV)</th>
<th>Electric field intensity (kV/cm)</th>
<th>Pulse width (µs)</th>
<th>Maximum temp. (°C)</th>
<th>Inactivated vegetative cells (Log$_{10}$)</th>
<th>Inactivated ascospores (Log$_{10}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple</td>
<td>2.50 × 10$^3$</td>
<td>19.4</td>
<td>32.3</td>
<td>2.5</td>
<td>19</td>
<td>4.8</td>
<td>3.6</td>
</tr>
<tr>
<td>Orange</td>
<td>3.63 × 10$^3$</td>
<td>20.6</td>
<td>34.3</td>
<td>2.0</td>
<td>20</td>
<td>4.7</td>
<td>3.8</td>
</tr>
<tr>
<td>Grape</td>
<td>2.70 × 10$^3$</td>
<td>21.0</td>
<td>35.0</td>
<td>2.2</td>
<td>20</td>
<td>5.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Pineapple</td>
<td>5.10 × 10$^3$</td>
<td>19.8</td>
<td>33.0</td>
<td>3.3</td>
<td>22</td>
<td>4.3</td>
<td>3.4</td>
</tr>
<tr>
<td>Cranberry</td>
<td>1.05 × 10$^4$</td>
<td>21.9</td>
<td>36.5</td>
<td>3.3</td>
<td>22</td>
<td>4.6</td>
<td>4.2</td>
</tr>
</tbody>
</table>

**Table 2—Treatment conditions and inactivation of vegetative cells and ascospores of Z. bailii by PEF (30 kV input voltage, 2 pulses) in fruit juices**

**Fig. 1—Inactivation of vegetative cells (open symbols) and ascospores (closed symbols) of Z. bailii suspended in orange ($\mathbb{A}_3$), pineapple ($\mathbb{O}_3$), apple ($\mathbb{A}_8$), cranberry ($\mathbb{E}_8$), and grape ($\mathbb{O}_7$) juices by HHP (300 MPa, 25°C).**
similar to the resistance of the same strain resuspended in a 0.1 M citrate buffer adjusted to pH 4 (Pandya et al., 1995). No data about the HHP inactivation of Z. bailii ascospores has been published, though Previdi et al. (1994) determined that S. cerevisiae ascospores were more resistant than vegetative cells when treated at 400 MPa in a culture broth.

The treatment menstruum did not influence the HHP inactivation of the vegetative cells, but did have an impact on ascospore inactivation which was fastest in cranberry juice and slowest in grape juice. No differences were found between the HHP inactivation of ascospores in orange, apple, or pineapple juice. Although some studies (Iwashashi et al., 1994) have suggested that the damage produced on yeast by HHP may be similar to that produced by heat, the different influences of media on the heat and HHP inactivation of yeast does not support this hypothesis.

The HHP survival curves of the vegetative cells ended in a tail. This could indicate the presence of a low number of ascospores (1 every 10⁷ vegetative cells) in the vegetative cell suspension, but a variation in resistance of individual cells of the Z. bailii population could also be responsible. Some researchers have reported a two-phase survival curve of non-populated microorganism vegetative cells inactivated by HHP (Cheftel, 1995).

The PEF treatment conditions for the different fruit juices and PEF resistance of Z. bailii ascospores and vegetative cells were also compared (Table 2). Applied electric field and pulse width are important factors influencing microbial inactivation by PEF. Peak voltage at the treatment chamber and gap between the electrodes determine the applied electric field.

The input voltage used in all treatments was constant (30 kV), but the peak voltage at the treatment chamber was related to the type of fruit juice (Table 2). Pulse width was also dependent on the fruit juice and was longer in those of lower conductivity (Table 2).

PEF treatment was very effective in inactivating both vegetative cells and ascospores. Two pulses with an electric field between 32-36.5 kV/cm decreased the population of vegetative cells between 4.5-5 and ascospores 3.5–4 log cycles (Table 2). However, when vegetative cells were inactivated no yeast was detected after three pulses, but the inactivation curves of the ascospores did end in a tail. This tail represented between 0.1–0.01% of the population and tended to disappear when the number of pulses was increased. This change in the ascospore suspension could be explained if a mixed population of ascospores (0.1–0.01%) and vegetative cells (99.9–99.99%) was in the suspension. However, microscopic observations of the suspension showed that more than 90% of the population was ascospores.

It is not highly reliable to compare the results obtained from different studies due to the different equipment and treatment conditions, but in general, results reported by others have shown that vegetative cells of yeasts are the microorganisms most sensitive to PEF treatment (Grahl and Markl, 1996; Qin et al., 1995d). No data to compare the inactivation of yeast ascospores by PEF were found.

Yeast ascospores like bacterial spores are more resistant to adverse physical and chemical agents than corresponding vegetative cells. However, yeast ascospores are much less resistant than bacterial spores. There are also some differences in structure and chemical composition between yeast spores and bacterial spores. Bacterial spores are smaller than yeast ascospores and the ascospore protoplast has a membranous structure that is nucleus, endoplasmatic reticulum or mitochondrial. Ascospores are higher in carbohydrate and lipid content and do not have dipicolinic acid (Fowell, 1975), but the lower resistance of yeast ascospores is probably due to no cortex in the ascospore. The cortex is considered important in bacterial spore heat resistance because it maintains the state of osmotic dehydration of the protoplast (Marquis and Shin, 1994). It is likely that the cortex is also important in the PEF resistance of bacterial spores reported in other studies (Pagan et al., 1997). Though the yeast ascospore walls may be important in the heat and HHP protection of ascospores, they did not protect most of the ascospore population against the PEF treatment in our study.

CONCLUSIONS
ALTHOUGH ASCOSPORES OF Z. BAILII HAD considerably higher heat resistance than vegetative cells, considering the z values (4–7) of the yeast ascospores reported in other studies, they should not present a problem in the preservation of pasteurized fruit juices. The presence of Z. bailii ascospores must be considered when calculating pressure treatment to assure the stability of fruit juices. Most of the Z. bailii ascospore population in this case was more resistant to PEF than vegetative cells, but more research is necessary to determine if it would be possible to achieve complete inactivation of the ascospore population.

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