Growth of Microorganisms in Chilled Orange Juice

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ABSTRACT

Growth of lactic acid bacteria and yeasts in chilled orange juice was investigated. Three suspensions were prepared, each consisting of four strains of Lactobacillus, four of Leuconostoc, and four of yeast. Each composite suspension was inoculated into a series of bottles containing sterile prechilled orange juice to obtain a final concentration of 1,100, and 1,000 organisms/ml. Samples were stored at 35, 40, 45, and 50 F (1.7, 4.5, 7.2, and 10 C), and were plated periodically throughout the test period. Yeasts grew at all temperatures investigated, their rates of growth increasing with the temperature. Lactobacillus organisms grew at 50 F but not at 45 F or below. Leuconostoc strains did not grow at 35 F, but grew slowly at 40 F, and rapidly at 45 F and 50 F. Fermentation by yeasts, depending on the level of inoculation, occurred in 1 week or less at 50 F and in 1 to 2 weeks at 45 F. At 35 and 40 F it occurred in 3 weeks but was satisfactory at 35 F for the lowest level of inoculation. Spoilage from growth of Lactobacillus was detected between 1 and 2 weeks at 50 F. It did not occur at 45 F or below. Leuconostoc required 13 days to 5 weeks at 40, 45, and 50 F. Spoilage did not occur at 35 F. Shelf life of chilled orange juice is dependent upon the initial microbial population at time of packaging and temperature maintained until it reaches the consumer.

There has been in recent years an increasing demand by the general public for convenience-type foods. Chilled orange juice (COJ) is no exception. In the past 10 years consumption of this product has increased from 27.3 million gal. in 1962-63 season to 112.4 million gal. in 1972-73 (5). To continue this phenomenal growth the quality of the product must be maintained from the producer to the customer.

Chilled juice is a broad class of product sold in single strength form usually refrigerated at the retail level. It may be packed as a sterile or unsterile product and it may contain preservatives. In the non-sterile form it is subject to microbial growth and spoilage—the predominant microflora being yeast and lactic acid bacteria. This orange juice is generally prepared from frozen orange concentrate (FCOJ) by reconstituting with chilled water to the desired Brix level. The resulting product may or may not be pasteurized before packaging. Juice is usually filled into paper cartons, or glass or plastic jugs at a temperature of 35 F or less. The level of contamination at the time of filling has a direct bearing on the ultimate shelf life of the product. The purpose of this study was to determine the growth of yeast and lactic acid bacteria that might be present in marketing non-sterile chilled juice at temperatures normally encountered from the producer to the customer.

RESULTS AND DISCUSSION

Growth and survival curves prepared from viable count data for initial inoculum levels of 1 and 1,000 organisms/ml are shown in Fig. 1-3. Generation times are presented in Table 2.
Arrows in the figures indicate when spoilage was first detected at time of plating. The times noted are not precise as spoilage could have occurred just after the previous plating when the product was found to be satisfactory. The samples were generally plated at intervals ranging from 2 to 5 days.

**Yeast and lactic acid bacteria**

Yeast grew at all temperatures investigated (Fig. 1). They grew progressively faster as the temperature was increased from 35 to 50°F, and the time to produce spoilage decreased accordingly as the level of inoculum increased (Table 1). The yeast appeared to grow slowly at 35 and 40°F but decidedly faster at 45 and 50°F.

Fermentation by yeasts, depending on the level of inoculum, occurred in 1 week or less at 50°F and in 1 to 2 weeks at 45°F. At 35 and 40°F it occurred in 3 weeks but was satisfactory at 35°F for the lowest level of inoculation.

Both *Lactobacillus* and *Leuconostoc* strains died at 35°F regardless of the level of inoculum (Fig. 2 and 3).

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Table 1. Effect of temperature and level of inoculum of test organisms on development of spoilage in orange juice

<table>
<thead>
<tr>
<th>Temp. (°F)</th>
<th>Yeasts 1/ml</th>
<th>1000/ml</th>
<th>Lactobacillus 1/ml</th>
<th>1000/ml</th>
<th>Leuconostoc 1/ml</th>
<th>1000/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>NS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21</td>
<td>NG&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NG</td>
<td>35</td>
<td>27</td>
</tr>
<tr>
<td>40</td>
<td>21</td>
<td>21</td>
<td>NG&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NG</td>
<td>35</td>
<td>27</td>
</tr>
<tr>
<td>45</td>
<td>14</td>
<td>7</td>
<td>NG&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NG</td>
<td>35</td>
<td>13</td>
</tr>
<tr>
<td>50</td>
<td>7</td>
<td>2</td>
<td>14</td>
<td>12</td>
<td>20</td>
<td>13</td>
</tr>
</tbody>
</table>

<sup>a</sup>NS = No spoilage detected after 28 days.

<sup>b</sup>NG = No growth.
The *Leuconostoc* grew at a very slow rate at 40 F, having a generation time of 102 hrs. (Table 2). Berry et al. (2) reported *Leuconostoc* as well as *Lactobacillus* did not grow but died at this temperature. Our data indicate the *Leuconostoc* organisms remained more or less dormant at 40 F for 7 days, after which they grew at a slow rate, increasing at the higher level of inoculum from 1,000 to 10,000 organisms/ml in 14 days. Above 40 F the rate of growth increased with temperature. At 45 and 50 F the generation times were 42 and 15 h, respectively. (Table 2).

Spoilage, depending on level of inoculum, occurred in 13 days to 5 weeks at 40, 45, and 50 F. It was not detected at 35 F.

*Lactobacillus* strains did not grow at 45 F or below and spoilage was not detected. At 50 F they grew rapidly with a generation time of 9.6 h (Table 2), and spoilage was first detected at 12 days.

Data obtained for the test organisms when the product was inoculated at an initial level of 100 organisms/ml is not included as the results were similar to those from the other two levels of inoculum. A comparison of the growth of yeast and lactic acid bacteria in orange juice at 45 F is shown in Fig. 4.

There was no correlation between plate counts and spoilage. With yeasts it more or less depended upon the initial level of inoculum and the incubation temperature. However, with the lactic acid bacteria some interesting assumptions can be drawn. One of the end products produced by these organisms in diacetyl which in orange juice produces off-flavors described as being similar to flavor of buttermilk (6, 7). It appears where growth occurred the microbial population found to produce spoilage decreased as the temperature was lowered -9.7 million at 50 F versus 22,000 at 40 F (Table 3).

### Table 2. Generation times (h) of yeast, *Leuconostoc*, and *Lactobacillus*

<table>
<thead>
<tr>
<th>Organism</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35 F</td>
</tr>
<tr>
<td>Yeast</td>
<td>19.6</td>
</tr>
<tr>
<td><em>Leuconostoc</em></td>
<td>NG</td>
</tr>
<tr>
<td><em>Lactobacillus</em></td>
<td>NG</td>
</tr>
</tbody>
</table>

NG = No growth.

### Table 3. Microbial population in orange juice when spoilage first detected

<table>
<thead>
<tr>
<th>Temp. (F)</th>
<th>Yeast</th>
<th><em>Lactobacillus</em></th>
<th><em>Leuconostoc</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>280,000</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>40</td>
<td>170,000</td>
<td>NG</td>
<td>22,000</td>
</tr>
<tr>
<td>45</td>
<td>270,000</td>
<td>NG</td>
<td>270,000</td>
</tr>
<tr>
<td>50</td>
<td>220,000</td>
<td>170,000</td>
<td>9,700,000</td>
</tr>
</tbody>
</table>

*Initial inoculum in juice = 100 organisms/ml.

NG = No growth.

true at all levels of inoculum studied. Christensen and Pederson (4) found that lactic acid bacteria produce greater quantities of diacetyl under less optimum conditions for growth. This might explain the results obtained in this study. They also reported that certain strains of lactic acid bacteria produce more diacetyl than others. Berry et al. (2) noted that of two *Lactobacillus* strains tested, one produced diacetyl at about 165 times the rate of other, although its growth rate was considerably slower. This would also indicate that off-flavors could be produced in orange juice with a relatively low microbial population.

At temperatures of 45 F or lower yeasts are the predominant flora responsible for spoilage in chilled orange juice. Lactic acid bacteria grow at a much slower rate or not at all. At 50 F or above these organisms could outgrow yeasts and be the cause of spoilage.

### Shelf life

The ultimate goal in processing chilled orange juice is to produce a product with extended shelf life. This is dependent, as the data indicate, on the initial level of contamination, the type of microflora present, and temperature of storage. Rushing and Senn (9) reported that the shelf life was significantly longer at 30 F (–1 C) than at 40 F (4.5 C). Patrick and Hill (8) noted product stored at 50 F (10 C) and 60 F (15.6 C) spoiled rapidly, but fermentation did not occur in juices held at 30 and 40 F for 3 weeks. They found temperature to be the most important factor in preventing microbial spoilage of chilled orange juice. Our data indicate not only temperature, but also the initial level of contamination play an important role. The higher the initial level the shorter the shelf life at a given temperature.
One source of temperature abuse occurs at the retail food stores. Barnard (1) in his study on the shelf life of milk found that of 250 samples of milk products checked in display cases nearly one-fourth of the samples were over 45°F with 42% in the range of 41 to 45°F. Bodyfelt and Davidson (3), in a similar study, found that of 352 samples checked 28% were over 45°F and 7% over 50°F. It is assumed the results they obtained would also be applicable to chilled orange juice.

It is evident that considerable temperature abuse occurs at the retail outlet. Therefore, one can conclude that to maintain extended shelf life of chilled orange juice it is imperative that the temperature and the microbial population of the product be kept to a minimum.

ACKNOWLEDGMENTS

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REFERENCES