GROWTH OF MICROORGANISMS IN CHILLED ORANGE JUICE

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Abstract. The growth of lactic acid bacteria and yeasts in chilled orange juice was investigated. Three suspensions were prepared, each consisting of 4 strains of Lactobacillus, 4 of Leuconostoc, and 4 of yeasts. Each composite suspension was inoculated into a series of bottles containing sterile prechilled orange juice so as to obtain a final concentration of 1, 100, and 1,000 organisms per ml. The samples were stored at 35°, 40°, 45°, and 50°F, and were plated periodically throughout the test period.

The yeasts grew at all temperatures investigated, their rates of growth increasing with the temperature. The 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 occurred in 1 week or less at 50°F and in 1 to 2 weeks at 45°F, depending on the level of inoculation. 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 12 days 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 the 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 the consumption of this product has increased from 27.3 million gals. in the 1962-63 season to 112.4 million gals. in 1972-73 (5). To continue this phenomenal growth the quality of the product must be maintained from the producer to the consumer.

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 prior to packaging. The juice is usually filled into paper cartons, 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.

Experimental Procedure

The test organisms used in this investigation consisted of: (1) 4 strains of yeast (Zygosaccharomyces vini and Z. rouxii) and 2 isolates from frozen concentrated orange juice); (2) 4 strains of Leuconostoc (2 of Leuconostoc sp. and 1 each of Leuconostoc mesenteroides and L. citrovorum); and (3) 4 strains of Lactobacillus sp.1

Each isolate was maintained for 3 weeks in sterile single strength orange juice (SSOJ) to insure active growth; then on Potato Dextrose Agar (PDA) slants prepared in 8 oz. screw cap bottles. Incubation was at 86°F (30°C) for 48 to 72 hrs. Growth on each slant was washed with sterile

1Received from Research Dept., Continental Can Co., Inc., Chicago, Ill.
2American Type Culture Collection (ATCC 8082 and 8293).
Fig. 1. Effect of temperature on the growth of yeast in orange juice inoculated to contain 1 and 1000 organisms per ml.
Fig. 2. Effect of temperature on the growth of Lactobacillus in orange juice inoculated to contain 1 and 1000 organisms per ml.
Fig. 3 Effect of temperature on the growth of Leuconostoc in orange juice inoculated to contain 1 and 1000 organisms per ml.
phosphate buffer (pH 7.1) into a sterile bottle containing glass beads for breaking up clumps of cells. A suspension was made in this manner for each test organism. Concentration of cells was determined by plating in quadruplicate and results averaged according to standard methods. Three composite suspensions were made from each group of test organisms (yeast, Lactobacillus, and Leuconostoc) by adding the individual suspensions together in such proportions that gave equal numbers of each strain. The resulting suspension was used immediately or stored in the refrigerator not to exceed 48 hrs.

The juice used in this study was prepared from reconstituted FCOJ, 12° Brix (pH 3.8). One thousand ml was placed into a series of 1/2 gal. glass screw cap bottles, heated to 180°F for 20 min. and then prechilled to desired incubation temperatures prior to inoculation. The juice was then inoculated with each composite suspension as follows: 4 bottles with 1,000 organisms per ml, 4 with 100 organisms per ml, and 4 with 1 organism per ml. The juice was incubated at 35°, 40°, 45°, and 50°F (1.7°, 4.5°, 7.2°, and 10°C). The samples were plated in duplicate immediately and at periodic intervals until spoilage was detected. Prior to each plating the bottles were placed on a mechanical shaker for 30 sec. Controls were prepared for each temperature and were plated and checked organoleptically for off-flavors at the same time the test samples were analyzed for microbial growth. Generation times were calculated as described by Berry, et al (2) from the expression:

\[ G = \frac{0.3(t_1 - t)}{\log N_1 - \log N} \]

Where \( G \) = Generation time in hours
\( N_1 \) = Number of orgs. at \( t_1 \)
\( N \) = Number of orgs. at \( t \)

Results and Discussion

Growth and survival curves prepared from Table 1. Generation times (hrs.) of yeast, Leuconostoc and Lactobacillus.

<table>
<thead>
<tr>
<th>Temp. °F</th>
<th>Yeast</th>
<th>Lactobacillus</th>
<th>Leuconostoc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Org.</td>
<td>35°F</td>
<td>40°F</td>
<td>45°F</td>
</tr>
<tr>
<td>Yeast</td>
<td>19.6</td>
<td>14.9</td>
<td>10.7</td>
</tr>
<tr>
<td>Leuconostoc</td>
<td>--</td>
<td>102.0</td>
<td>42.0</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Table 2. Effect of temperature and level of inoculation of test organisms on development of spoilage in orange juice.

<table>
<thead>
<tr>
<th>Temp. °F</th>
<th>Yeasts</th>
<th>Lactobacillus</th>
<th>Leuconostoc</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>NS</td>
<td>21</td>
<td>NG</td>
</tr>
<tr>
<td>40</td>
<td>21</td>
<td>21</td>
<td>NG</td>
</tr>
<tr>
<td>45</td>
<td>14</td>
<td>7</td>
<td>NG</td>
</tr>
<tr>
<td>50</td>
<td>7</td>
<td>2</td>
<td>14</td>
</tr>
</tbody>
</table>

NS = no spoilage detected after 28 days
NG = no growth

Viable count data for initial levels of inoculation of 1 and 1,000 orgs./ml are shown in Figs. 1 through 3. Generation times are presented in Table 1.

Arrows in the figures indicate when spoilage was first detected at time of plating. The times noted are not precise as it 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 increased from 35°F to 50°F, and the time to produce spoilage decreased accordingly as the level of inoculation increased (Table 2). The yeast appeared to grow slowly at 35° and 40°F but decidedly faster at 45° and 50°F. Fermentation by yeasts occurred in 1 week or less at 50°F and in 1 to 2 weeks at 45°F, depending on the level of inoculation. At 35° and 40°F it occurred in 3 weeks but was satisfactory at 35°F for the lowest level of inoculation.

Both the Lactobacillus and Leuconostoc strains died at 35°F regardless of the level of inoculation (Fig. 2 and 3). The Leuconostoc grew at a very slow rate at 40°F, having a generation time of 102 hrs. (Table 1). 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 inoculation from 1,000 to 10,000 organisms per ml in 14 days. Above 40°F the rate of growth increased with temperature. At 45° and 50°F the
Fig. 4. Growth of yeast and lactic acid bacteria in orange juice stored at 45°F.
generation times were 42 and 15 hrs. respectively (Table 1). Spoilage, depending on level of inoculation, occurred between 13 days and 5 wks at 40°, 45° and 50°F. It was not detected at 35°F.

The 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 hrs. (Table 1), 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 per ml is not included as the results are similar to the other 2 levels of inoculation. However, as a matter of interest 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. In the case of yeasts it was more or less dependent upon the initial level of inoculation 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 is diacetyl which in orange juice produces off-flavors described as being similar to 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). This was true at all levels of inoculation 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 the other, although its growth rate was considerably slower. This would also indicate 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 out 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 out of 352 samples

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

<table>
<thead>
<tr>
<th>Initial Juice Level of Inoculation 100 org. per ml</th>
<th>Yeast</th>
<th>Lactobacillus</th>
<th>Leuconostoc</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temp. °F</strong></td>
<td>Microorganisms per ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</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>

NG = no growth

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**Table 4. Temperature of 602 milk product samples in display cases, Oregon and Pennsylvania retail food stores.**

<table>
<thead>
<tr>
<th>Range</th>
<th>Oregon</th>
<th>Pennsylvania</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>40°F or less</td>
<td>117</td>
<td>33.2</td>
</tr>
<tr>
<td>41 to 45°F</td>
<td>135</td>
<td>38.4</td>
</tr>
<tr>
<td>Over 45°F ²</td>
<td>100</td>
<td>28.4</td>
</tr>
</tbody>
</table>

²Samples in excess of 50°F = 7.4%

YFrom Bodyfelt and Davidson, 1974

XFrom Barnard, 1974
checked 28% were over 45°F and 7% over 50°F (Table 4). It is assumed the results they obtained would also be applicable to chilled orange juice.

It is evident a considerable temperature abuse occurs at the retail outlet. Therefore, one can conclude in order 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.

Literature Cited

Effects of UV-B Radiation on Pigment Changes and Quality of Citrus Fruits

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Abstract. Effects of UV-B radiation (290-310 nm) on color changes and fruit quality of 'Hamlin' sweet orange (Citrus sinensis (L.) Osbeck) were tested. UV-B radiation had a destructive effect on the total chlorophyll in the rind. The rate of chlorophyll degradation was the highest in fruits treated with 1 x 10³ ppm 2-chloroethane-phosphonic acid (ethephon) and exposed for 30 days to UV-B radiation. Treated fruits significantly reflected a greater proportion of visible light than control fruits or fruits treated with GA and kept under light conditions minus UV-B or in the dark. It is conceivable that the mechanisms of carotenoid synthesis, already present in the rind appeared to be favored by UV-B radiation. Samples treated with UV-B were predominantly yellow and expressed a gradual decline in Hunter color difference meter aL/bL ratios. UV-B radiation had an indirect effect on internal quality of 'Hamlin' orange through its action on external qualities of the fruit.

Solar ultraviolet radiations constitute a separate and distinct environmental factor that produces a number of effects on living organisms. These effects are numerous and can be deleterious to plants. These range from darkening of surface tissues to complete inhibition of growth and subsequent death of the plant or plant parts (6, 7, 10). There is considerable variation in the reaction of plants to UV radiation depending on latitude, season, variation in temperature, overall light intensity, wavelength, daylength and others. The interaction between the plants and UV radiation is influenced by the complexity of ecological conditions, relative intensity and wavelength of solar UV radiation entering the biosphere which varies strongly with time and locality. Regions at lower latitudes receive up to 400% more UV radiation than regions at higher latitudes.

Much concern has been expressed recently over the effects of solar UV radiation in Florida because of the lower latitude and the longer growing season. Citrus is the major industry in Florida. Total citrus production is approximately 200,000,000 boxes of fruits produced from about 1,000,000 acres. About 7% of the total orange production goes annually to the fresh market for local consumption. Both external and the internal color of the fruits are important factors influencing consumer preference for fruits. Despite the fact that the color alone is not a reliable indication of maturity, the consuming public generally judges the maturity of citrus fruits by their color.