Effect of Temperature on Survival of Yeast in 45° and 65° Brix Orange Concentrate

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ABSTRACT

A study was conducted to determine the effect of cold-temperature storage on yeast survival in 45° and 65° Brix orange concentrate, under simulated conditions of bulk storage. Suspensions were prepared from each of three strains of yeast which had been identified as to genus and species. Orange concentrates at 45° and 65° Brix were inoculated to contain approximately 1,000,000 organisms per ml. Inoculated samples were then stored at -17.8, -9.4, -1.1, and 4.4 C. Samples were analyzed for total viable yeast periodically during 15 months of storage. No yeast growth occurred in either 45° or 65° Brix concentrate at temperatures below 4.4 C. Survival curves of one of the test organisms in both 45° and 65° Brix are presented. Yeast died faster in 45° Brix at -17.8° C than at -9.4 or -1.1 C, while in 65° Brix survival was greater at lower temperatures. Mold was detected in 45° Brix at -1.1 C and below after 7 or more months. In 65° Brix, mold was observed after 12 months at all temperatures above -17.8 C. Some samples showed a slight brown discoloration after extended periods of storage.

It has been the general practice in the citrus industry to store bulk product in 55-gal. drums at -17.8 to -23.3 C. This has involved the use of thousands upon thousands of drums each season. Product in drums requires a considerable amount of handling-filling, storage, when removed from the warehouse, thawing, and again when the product is blended back into the concentrate stream - not a very efficient operation. As consumption of frozen orange concentrate increased, it became apparent that a more practical method was needed to handle bulk product. As a result, large stainless steel tanks have been built, some of which have a capacity of over 100,000 gal. They are housed in refrigerated warehouses, the size of the buildings depending upon the number of tanks they will accommodate. The tanks are filled with orange concentrate, usually 65° Brix, at temperatures which may range at some plants from -9.4 C to as high as -1.1 C. Periods of storage could range from a few months to over a year. Little is known of what metabolic activity may take place under these storage conditions. Kitchel (3) investigated the survival of four different strains of osmophilic yeast in 60° Brix orange concentrate at -15, -6.7, and 4.4 C. All strains grew at 4.4 C but not at -6.7, or -15 C. Murdock and Dubois (5) investigated growth of four strains of osmophilic yeast in 58.5° Brix concentrate. They reported growth at 4.4 C but not at -9.4 and -17.8 C. In 70° Brix concentrate no growth occurred at 4.4 C. Yeast have been reported to grow in other fruit products at temperatures below 0 C. Pederson et al. (7) found a Candida sp. to grow at -2.2 C in grape juice. Berry and Magoon (1) reported Torula sp. to grow in berries at 40% sucrose at -4 C.

This study was conducted to determine the effect of cold-temperature storage on yeast survival in 45° and 65° Brix orange concentrate, under simulated conditions of bulk storage.

EXPERIMENTAL PROCEDURE

Test organisms used in this investigation were three strains of yeast identified as Zygossaccharomyces vini (Y-35), Z. rouxii (Y-36), and Hanseniaspora melligeri (Y-10). According to Lodder (4) the first two strains have been classified as Saccharomyces rouxii and the third, Hanseniaspora valbyensis, being synonymous with H. melligeri. Suspensions of each strain (hereafter referred to as A, B, and C, respectively) were prepared by washing growth from Potato Dextrose Agar slants with sterile distilled water. The concentration of each suspension was determined by the agar plate method using Orange Serum Agar containing 5% sucrose. These suspensions were then used to inoculate 45° and 65° Brix orange concentrate so that each contained approximately 1,000,000 organisms per ml. The appropriate amount of strain A suspension was added to the concentrate and mixed in a Waring Blender for 2 min. Inoculated concentrate was then transferred to sterile test tubes (approximately 5 ml. per tube.) The same procedure was repeated with suspensions of yeast strains B and C. Approximately 40 replicate tubes of each variable were then placed in cold storage for each temperature investigated (-17.8, -9.4, -1.1, and 4.4 C). Duplicate samples of each variable were removed at various intervals over a 15-month period and analyzed for total viable count. Each sample was plated in duplicate, using Orange Serum Agar. Plates were counted after 48-72 hr of incubation at 30 C.

The 65° Brix orange concentrate was prepared from 67° Brix evaporator pump-out by adding sufficient sterile water to obtain the desired Brix. Commercial product was used as the source of 45° Brix concentrate. Brix / acid ratios for 45° and 65° Brix concentrate were 14.6 and 19.0.

RESULTS AND DISCUSSION

Survival of yeast strain A in 45° and 65° Brix concentrate at -17.8, -9.4, -1.1, and 4.4 C is shown in Fig. 1 and 2. Survival curves for strains B and C were quite similar to strain A, therefore they are not shown.
Yeast did not grow in 45° Brix concentrate at any of the temperatures investigated below 4.4 C. At this temperature, one strain grew in 2 months and the other two strains in 3 months. Mold was detected in 7 months at -1.1 C and 12 months at -17.8 and -9.4 C. A slight brown discoloration was noted at 7 months at -1.1 C but not at -17.8 and -9.4 C.

None of the yeast cultures grew in 65° Brix concentrate even when held for extended periods at 30 C. However, it is known that certain strains of osmophilic yeast will grow in 65° Brix concentrate. The Research Department of Continental Can Company, Inc. (2) investigated yeast spoilage in 65° Brix orange concentrate. Since our data showed no growth in 45° Brix concentrate at -1.1 C it is believed this would also be true in 65° Brix concentrate even if we had a yeast that would grow at this concentration. Mold growth was noted at 9 months at -1.1 C and 12 months at -9.4 and 4.4 C but not at -17.8 C. A slight discoloration was observed at -1.1 and 4.4 after 15 months, but not at -17.8 or -9.4 C.

The number of months required to reduce the yeast population by 90% is shown in Table 1. It is noteworthy that all three strains died faster in 65° Brix concentrate at 4.4 C than they did at the other temperatures investigated. Except for strain B, the yeast did not die as fast at -17.8 C as they did at the other three temperatures. Generally speaking, it appears that the lower the temperatures the slower the death rate. However, in 45° Brix concentrate this trend was not evident. At -9.4 C the yeast survived in larger numbers than they did at either -17.8 or -1.1 C.

Even though our data showed no microbial growth, metabolic activity still takes place in the viable cells, the end products of which could possibly produce off-flavors. Murdock and Brokaw (6) noted 6-oz. cans of 42° Brix orange concentrate held at 4.4 C swelled and sometimes burst when no increase in microbial population occurred. In this particular case, respiration of yeast apparently produced enough gas to cause cans to swell and sometimes burst. It is not known whether this evolution of gas could possibly be a problem after long periods of storage of bulk concentrate.

**Effect of temperature change on yeast population**

It is the custom in the citrus industry to hold high-count product in the freezer until the total viable count reaches an acceptable level. Sometimes this may occur after a few weeks, several months, or it may never reach acceptable levels. Data presented herein show two of the three strains died slower in 65° Brix concentrate at -17.8 C than they did at the other three temperatures investigated. The effect of temperature on yeast population is shown in Table 1.

**Table 1. Number of months to reduce yeast population level 90%**

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>45° Brix Strain</th>
<th>65° Brix Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>-17.8</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>-9.4</td>
<td>&gt;15</td>
<td>8</td>
</tr>
<tr>
<td>-1.1</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>4.4</td>
<td>G</td>
<td>G</td>
</tr>
</tbody>
</table>

*aG = Growth.*
change on yeast population in 65° Brix orange concentrate was further investigated. One, designated as a laboratory study, involved inoculating 65° Brix orange concentrate with yeast strain A, placing the inoculated material into a series of duplicate test tubes, holding 1 set for 2, 4, and 6 days at -1.1 C and another group for the same length of time at 4.4 C, then placing tubes at -17.8 C for 1 week, after which the product was plated for total viable yeast count. The results in Table 2 show the greatest reduction in yeast population occurred when the product was held 2 days at either -1.1 or 4.4 C. Holding it for longer periods at these temperatures did not appear to have any beneficial effect.

**TABLE 2. Effect of temperature change on yeast population in 65° Brix orange concentrate.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Laboratory Study Products held 2, 4, 6 days at -1.1 and 4.4 C, then plated after 1 wk. at -17.8 C.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days</td>
</tr>
<tr>
<td></td>
<td>1.1 C</td>
</tr>
<tr>
<td>2</td>
<td>630</td>
</tr>
<tr>
<td>4</td>
<td>550</td>
</tr>
<tr>
<td>6</td>
<td>450</td>
</tr>
</tbody>
</table>

In another investigation, 15 5 gal. drums of 58° Brix orange blend were removed from -17.8 C storage and held 2 days at room temperature (product temperature 10-15.6 C) and then 2 weeks at -17.8 C. Yeast population before treatment ranged from 240-3,000 per ml, and after, 12-825 - a sizable reduction. The results represent what might occur with a mixed yeast flora, as this was commercial product. Also, no flavor degradation was noted as a result of this treatment.

**SUMMARY**

In summary, our data indicated: (a) Yeast did not grow in 45° Brix concentrate at any temperature investigated below 4.4 C. At this temperature one strain grew in 2 months and the other two in 3 months. (b) Mold was detected in 45° Brix concentrate after 12 months at all temperatures investigated below 4.4 C. A slight brown discoloration was noted after 7 months at -1.1 C but was not evident in -9.4 or -17.8 C samples. (c) Yeast did not grow in 65° Brix concentrate at any temperature during extended storage. (d) Mold growth was noted in 65° Brix concentrate after 12 months at all temperatures investigated above -17.8 C. A slight discoloration was observed at -1.1 and 4.4 C after 15 months but not at -9.4 or -17.8 C.

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**REFERENCES**