In the marketing of frozen citrus concentrates there has been chronic occurrence of customer complaints due to can explosions. Complaints of this type, fortunately, have been exceedingly low—averaging less than 1.5 per 10,000,000 units of 6 and 12-ounce cans of frozen concentrated orange juice sold according to a survey made by one manufacturer (7). The persistent occurrence of this type of complaint led to an investigation to determine the degree of abuse at 40° F., and room temperature required to produce can swells and can explosions in frozen concentrated orange juice. The preliminary results of this study were presented at the 1961 Annual Meeting of the Florida State Horticultural Society. Data indicate that in order for cans of frozen concentrated orange juice to swell and/or explode, the product must have received a considerable amount of abuse in terms of temperatures at 40° F., or above.

Since the previous paper dealt only with product representative of the 1961 midseason pack, it was decided to investigate the effect of exposing frozen concentrated orange juice to adverse storage conditions with samples representative of the 1961 Valencia season, and both midseason and Valencia portions of the 1961-62 pack. By so doing, it was thought the results would be more meaningful since considerably more product would be under test. It was also decided to study more critically the microbial population in orange concentrate at both 40°F. and room temperature.

**PROCEDURE**

Samples for this study were collected and examined in the same manner as described in our previous publication (11). As a brief review, twelve 6-ounce cans of frozen concentrated orange juice were collected from the freeze tunnel at the end of each evaporator run which during the investigation ranged from 60-72 hours. The cans
TABLE I

SPOILAGE DEVELOPING AT ROOM TEMPERATURE IN 6-OZ. CANS OF ORANGE CONCENTRATE

Product Representative of Two Citrus Seasons

<table>
<thead>
<tr>
<th>Season</th>
<th>No. Cans Tested</th>
<th>No. Days All Cans Flat</th>
<th>Days Required For 1 or More Cans to Swell</th>
<th>Days Required For 1 or More Cans to Burst</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. Days</td>
<td>% Swelled</td>
</tr>
<tr>
<td>1961 Mid.(1)</td>
<td>150</td>
<td>2</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>1961 Valencia</td>
<td>100</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>1962 Mid.</td>
<td>100</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>1962 Valencia</td>
<td>50</td>
<td>3</td>
<td>4</td>
<td>82</td>
</tr>
</tbody>
</table>

Temp. Range: 70-74°F.
(1) Data from initial investigation.

were coded and placed immediately at 0 to —10°F. Four to 8 hours after the plant was again in operation following a clean-up a similar set of samples was obtained. All samples were collected from one plant and this sampling procedure was adhered to during the 1961 Valencia pack as well as during the entire 1961-62 citrus season. The number of cans collected each season, the period covered, and the weeks they were held in cold storage prior to test are as follows:

<table>
<thead>
<tr>
<th>Season</th>
<th>No. Cans Collected</th>
<th>Weeks Samples Held at 0 to —10°F. Prior to Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1961 Valencia</td>
<td>372</td>
<td>5 - 14</td>
</tr>
<tr>
<td>1962 Midseason</td>
<td>200</td>
<td>10 - 18</td>
</tr>
<tr>
<td>1962 Valencia</td>
<td>240</td>
<td>5 - 21</td>
</tr>
</tbody>
</table>

TABLE II

SPOILAGE DEVELOPING AT 40°F. IN 6-OZ. CANS OF ORANGE CONCENTRATE

Product Representative of Two Citrus Seasons

<table>
<thead>
<tr>
<th>Season</th>
<th>No. Cans Tested</th>
<th>No. Weeks All Cans Flat</th>
<th>Weeks Required For 1 or More Cans to Swell</th>
<th>Weeks Required For 1 or More Cans to Burst</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. wks.</td>
<td>% Swelled</td>
</tr>
<tr>
<td>1961 Mid.(1)</td>
<td>150</td>
<td>4</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>1961 Valencia</td>
<td>100</td>
<td>4</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>1962 Mid.</td>
<td>100</td>
<td>4</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>1962 Valencia</td>
<td>50</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

* Cans not checked to determine time required to burst.
(1) Data from initial investigation.
Three separate studies were made—one for each of the above seasons.

All product representative of a given pack was removed from cold storage at one time. Sub-samples were then selected so that the 50 to 100 cans of concentrate placed at room temperature (70-74°F.) contained product before and after each clean-up. These were examined visually for swells and can explosions. A duplicate group was placed at 40°F. to be similarly examined. Another replicate set of samples was set aside for microbiological examination at each of these two temperatures.

Product held at room temperature for visual examination was checked daily over a 5-day period. In addition, each day during this interval a 10-can sample representative of the entire pack was plated in duplicate on orange serum and acidified malt agar (pH 3.5).

Concentrate held at 40°F. was examined weekly for swells, at which time a 10-can cross-sectional sample was also plated on the media just described. This phase of the experiment was made over a 10-week period.

Just prior to plating, the 42° Brix concentrate samples were reconstituted to 12° Brix (single strength) juice and all counts were reported on this basis. Orange serum agar was used to determine the total viable microbial population of the product. It was also used as a differential medium for gum-forming and pinpoint type colonies which were counted and recorded separately. Yeast population was enumerated from the malt agar plates. Samples collected during the 1962 Valencia season were also plated on McClesky's agar (5). This medium was used because of its specificity for the growth of gum-forming organisms. All plates were examined after 72-96 hours of incubation at 30°C. (86°F.). The count recorded was the average of each set of duplicate plates.

On McClesky's agar only the gum-forming organisms were recorded. The graph shows the distribution of total viable organisms, yeast, gums, and pinpoint type colonies over a period of 6 weeks at room temperature (70-74°F.).

**Figure 1.**

**Microbial Population in 6-Oz. Cans of Orange Concentrate Stored at Room Temperature.**

- **Product Representative of 1961 and 1962 Valencia Seasons.**
- **Total Viable Organisms**
- **Yeast**
- **Pinpoint-Type Colonies**
- **Gum-Forming Organisms**
- **Cans Flat When Plated**
- **% of Cans Swelled or Burst When Plated**

Days at Room Temp. (70-74°F.)
colonies were counted. Representative gum-forming colonies were picked from each plate and streaked on orange serum agar slants for future reference. The catalase reaction of each gum-forming colony was determined, according to a procedure described by Murdock et al. (10), which consisted of flooding the plate with a 3% solution of hydrogen peroxide. If effervescence occurred the colony was considered "catalase positive." If no reaction, the colony was considered "catalase negative." The isolated cultures were inoculated into sterile, single strength orange juice (pH 3.8). If growth occurred as evidenced by a microscopic examination of the juice they were considered potential "off-flavor" producing organisms. The Voges-Proskauer (V.P.) test was made by growing the organisms in sterile orange juice, then adding alphanaphthol and KOH creatine reagents to the culture. A positive reaction was characterized by an intense pink color which developed in a few minutes, indicating the presence of acetylmethylcarbinol and/or diacetyl.

All counts from each 10-can sampling period were converted into logarithms, averaged, and the antilogs recorded. The concentrates used for this study had an average of 41.9° Brix, citric acid ranged from 2.28 to 3.20%, and Brix/acid ratio from 13.0 to 18.4:1. The pH ranged from 3.7 to 3.8.

RESULTS

Spoilage Developing at Room Temperature and 40°F.

Spoilage developing at room temperature in samples of 6-ounce cans of orange concentrate representative of 1960-61 and 1961-62 citrus seasons is presented in Table I. Table II shows results obtained when a similar set of samples were stored at 40°F. Data show concentrate removed from cold storage did not spoil when held for 2 days at room temperature, at which time...
all cans remained “flat” (that is, the can ends were not swelled). For product to swell and/or burst, 3 or more days were required. At 40°F., all cans of concentrate were still flat after 4 weeks. Five or more weeks were required for cans to swell. Additional results are shown in Tables I and II.

It is interesting to note that results obtained during this study reconfirm our findings obtained during the midseason portion of the 1961 pack. These data have been incorporated in Tables I and II to show this comparison.

Microbiological Results at Room Temperature

An examination of the data showed the microbial population in 6-ounce cans of orange concentrate stored at room temperature, representative of the 1961 Valencia season, compared closely with results obtained during the 1962 Valencia pack. Therefore, for sake of brevity, data from these two seasons have been combined. A graphical presentation of the results is presented in Figure 1. The data show that the total viable population consisted mostly of yeast after the 4th and 5th day. This was also reported in our previous paper when an examination of orange serum agar plates showed a majority of the colonies present to be yeast (11). These organisms grew rapidly in the product after the first day of the test period.

The pinpoint colonies, which are characteristic of the colonies produced by lactobacilli (9), and the gum-formers showed a reduction in viable numbers during the first 3 days followed by a slight increase in population at the end of the 5-day test period.

Figure 2 is a plot of the gum-forming organisms shown in Figure 1, separated in accordance with their catalase reaction. It will be noted that the gum-forming population is made up principally of catalase positive organisms. In
fact, 99% of the gum-formers present at the start of the test period gave a positive reaction when tested with hydrogen peroxide. It should be pointed out that it is the catalase negative organisms which produce diacetyl in orange juice and concentrate, the flavor of which has been described as being similar to “buttermilk” (3, 4, 8). It is this group of organisms along with those belonging to the genus Lactobacillus which are of sanitary significance in the production of frozen concentrated orange juice (13). Catalase positive gum-forming organisms have never been implicated in the production of off-flavors in orange juice or concentrate. However, a study of 38 catalase-positive cultures isolated throughout this investigation showed that 36 organisms grew in orange juice with the production of a variety of non-specific off-flavors, most of which were characterized as bitter or tart. All organisms were V.P. negative in orange juice indicating they do not produce diacetyl or acetyl-methylcarbinol.

It is suggested the large yeast population in the product between the 4th and 5th days of the test period is responsible for the slight increase in organisms producing pinpoint and gum-forming colonies. Yeast, in growing, utilize the soluble (sugar) solids in the concentrate as a source of energy. As the sugar solids are reduced from the original 42° Brix level, a point is reached where the lactic acid organisms may grow. Previous studies have shown that organisms belonging to the genera Leuconostoc and Lactobacillus grow very slowly or not at all at 42° Brix (12, 1). However, there is certainty of growth in the Brix range of 25-38° Brix or lower, with probable production of diacetyl (6).

**Microbial Population in Product at 40°F.**

Microbial population in 6-ounce cans of orange concentrate stored at 40°F. representative of the
1961 Valencia season is presented in Figure 3. Data show all organisms died in 42° Brix concentrate held at 40°F. during the first 6 weeks of the test period, after which there was an increase in yeast population. The gum-formers and organisms producing pinpoint type colonies, on the other hand, continued to show a reduction in numbers.

Another graph of the gum-forming organisms is shown in Figure 4, where those organisms giving a catalase positive and catalase negative reaction have been plotted. Of the gum-formers present, 88% were catalase positive and 12% catalase negative at the start of the test period. As indicated in Figure 3, a steady decrease in viable gum-forming organisms occurred throughout the test period.

Except for yeasts, the microbial population in all 6-ounce cans of orange concentrate stored at 40°F. gave results similar to those presented in Figures 3 and 4. The yeasts, however, showed a noticeable increase in numbers after 6 weeks in product representative of the 1962 midseason pack. Similar data were also obtained during the midseason portion of the 1961 pack which were presented in our previous paper (11).

In our original publication it was reported that a greater percentage of swells occurred at both room temperature and 40°F. in samples collected after, rather than before, each clean-up (11). In examining our present data it was noted there was a greater yeast population after, rather than before, each clean-up. A graph showing this relationship made from samples stored at 40°F. representative of the 1961 Valencia season is presented in Figure 5. It seems logical to assume that the occurrence of can swells in the 1961 pack is directly related to levels of yeast population as shown in the present studies.

![Graph showing yeast population in 6oz cans of orange concentrate at 40°F. representing product before and after clean-up from 1961 Valencia season.](image-url)
**Diacetyl Analyses**

Product representative of the 1961 portion of the Valencia pack stored at 40°F., and concentrate from the 1962 Valencia season held at room temperature (70-74°F.) were checked throughout the test period for diacetyl in accordance with the procedure described by Byer (2). Results which are presented in Figure 6 show no increase in diacetyl in product stored at 40°F., while at room temperature a noticeable increase occurred between the 3rd and 5th days. It is believed this is due to the activity of lactic acid organisms which was mentioned previously.

Based on data representative of the 1960-61 and 1961-62 citrus seasons, it is evident that in order for cans of frozen concentrated orange juice to swell and/or explode they must be held for 4 or more weeks at 40°F., 3 or more days at room temperature, or intermediate temperatures. When product is subjected to these adverse conditions it is the yeasts, rather than lactic acid organisms, which are responsible for spoilage.

As stated in our previous paper, it seems unlikely the customer would hold product in a refrigerator for such an extended period. Therefore, can swells or explosions, when reported by the customer, must be the result of holding concentrate at temperatures above 40°F.

**Summary**

Six-ounce cans of frozen concentrated orange juice were collected, before and after each clean-up, from one plant during the Valencia portion of the 1960-61 citrus season and again throughout the following season. Representative samples from each lot were held at both room temperature (70-74°F.) and 40°F. Samples were examined visually for swells and can explosions. They were also checked for total viable organisms, yeasts, gum-formers, and pinpoint colonies at periodic intervals throughout the test period.

At room temperature all cans of concentrate remained flat and showed no evidence of spoilage 2 days after removal from cold storage. For
product to swell and/or burst, 3 or more days were required. At 40°F., no swells developed at the end of 4 weeks. Five or more weeks were required for spoilage to occur.

Yeast were the only organisms which grew rapidly in product stored at room temperature. Bacteria producing pinpoint and gum colonies died off rapidly in concentrate during the first 3 days of the storage period. Between the 4th and 5th day there appeared to be a slight increase in the population of these organisms. During this period there was also a noticeable increase in the concentration of diacetyl in the product.

Except for yeasts, no microbial growth occurred in 42°F. Brix orange concentrate stored at 40°F. All microflora showed a reduction in numbers the first 6 weeks of the test period, after which there was a noticeable increase in yeast population. Organisms producing pinpoint and gum colonies continued to die throughout the 10-week storage period. The absence of growth of these organisms was reflected in the concentration of diacetyl in the product which remained, more or less, constant.

Gum-formers found in frozen orange concentrate were predominantly catalase positive, a group of bacteria which do not produce diacetyl or acetylmethylcarbinol in orange juice or concentrate.

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LITERATURE CITED


