Effect of Varied Storage Temperatures on Microorganisms in Frozen Concentrated Orange Juice

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Extensive studies on the effects of conditions that may be encountered in commercial handling on quality of frozen foods have been under way at the Western Utilization Research and Development Division of the U. S. Department of Agriculture during recent years. The commodities investigated have included frozen concentrated orange juice. Chemical and physical changes in this commodity under varied conditions of time and temperature have been reported by McColloch et al. (1957). The present paper deals with microbiological changes in 5 of the 9 lots of concentrate studied by McColloch and co-workers.

Although extensive research has been done on certain phases of the microbiology of citrus products, that published on the effect of varying storage temperatures on microorganisms in frozen concentrate is very limited. Faville et al. (1951) noted that bacteria were the predominate type of microbial flora in frozen concentrated orange juice and that these decreased rapidly in number when the product was stored at −17 C. They also observed that various microorganisms inoculated into orange concentrate were destroyed more rapidly at 40 C than at 30 or 3 C. The latter observations, however, were based on observations of periods from 1 to 7 hr and do not necessarily reflect what would happen in subfreezing storage for several months.

The phenomenon of storage temperature effect on other frozen foods has been observed by other workers. Berry (1932, 1933, 1936) observed repeatedly that greater destruction occurred in berry packs stored at 15 F (−9 C) than in those stored at −15 F (−26 C). Similar observations were made by Prescott et al. (1932), who found that in certain foods higher storage temperatures resulted in more rapid microbial destruction.

**Experimental Methods**

*Orange concentrates used.* The concentrates included 5 lots manufactured in California during the 1952 and 1953 seasons. Each lot was obtained from the concentrate plant on the day it was produced and brought to the laboratory under conditions which prevented any temperature change en route. The lots were placed at 0 F immediately upon arrival at the laboratory.

*Simulated time-temperature patterns.* The time-temperature variations used were devised from thermograph records of actual frozen orange concentrate shipments, records of cold storage warehouses and retail cabinet temperatures, and other information supplied by representatives of the citrus and frozen foods industries. From this information it was possible to arrange schedules of exposure to various temperatures for various periods that represent mild to severe conditions during the following 5 stages: (I) producer's warehouse, (II) transportation, (III) warehousing, (IV) wholesale and retail marketing, and (V) home refrigeration. Although several extreme conditions were used, their inclusion is not to be taken as indication that they commonly occur in distribution of the product, or that the stages at which they were included are those in which abnormal conditions are most likely to be encountered. The temperature variations were as follows:

Stage I: 0 F for one month.

Stage II: The records observed showed 3 deviations, from mild to severe: (a) a constant temperature of 10 F for 10 days; (b) constant temperature of 10 F for 5 days followed by a steady rise in temperature to 20 F in 5 days; or (c) by a steady rise in temperature to 40 F in 5 days.

Stage III: 0 F for 6 months.

Stage IV: Three temperature deviations were found to be worthy of consideration: (a) a constant temperature of 10 F for 14 days; (b) a constant temperature of 10 F for 14 days interrupted after the 6th day by thawing to 60 F and immediate recooling to 10 F in 12 hr (corresponding to removing concentrate from a retail cabinet and holding at room temperature while the cabinet is defrosted); and (c) a constant temperature of 20 F for 14 days.

Stage V: The two most extreme temperature deviations likely to be encountered were 20 and 40 F for 7 days.

*Testing schedules.* The schedules employed were based on temperatures monitored by thermocouples.
embedded in cans of concentrate and connected to a multiple-point recorder. Abrupt changes from 0°F to higher levels and back to 0°F were accomplished in periods of 12 hr or less.

Each of the 5 lots was first held at 0°F for 1 month to simulate stage I. Samples from these lots were next exposed to the temperature patterns simulating stage II, and then to 0°F (stage III) for 6 months. The 3 variables in stage II followed by stage III were next subjected to stage IV’s 3 temperature variables. Samples of all combinations of variables through stage IV were subjected to one of the two temperature conditions chosen to simulate conditions in the household refrigerator.

Controls were held at −10°F and at −80°F in a Dry Ice cabinet. Also, a small lot was stored at constant 40°F to follow the fate of microorganisms in the product when stored at this temperature.

**Bacteriological analyses.** All samples were plated on orange serum agar (Stevens, 1954) and plates were counted after incubation for 3 days at 86°F. A low-power, wide-field stereoscopic microscope was used to aid the counting. This microscope was used because particles of orange pulp were present on most plates and these could sometimes be mistaken for pinpoint colonies. It was not possible to make any actual identification of bacterial types from the plates, but observations were made of colony characteristics, especially of the mucoidal “Leuconostoc-type” colonies and those characteristic of yeasts.

Coliform determinations were made by methods previously described (Wolford, 1954), using both lactose broth and boric acid broth as presumptive media and carrying the analyses at least through the completed coliform tests.

**Results and Discussion**

Initial plate counts of the five lots of concentrate were: lot A, 176,000; lot B, 89,000; lot C, 290,000; lot D, 200,000; lot E, 220,000 per ml of concentrate. Coliforms were recovered from the initial samples of lots A, C, D, and E. Two of five samples of lot C analyzed at the start of the experiment had coliform indices of 39 per 100 ml of reconstituted juice; all other positives at this stage had MPN values of less than 10 per 100 ml. Only in lot C were coliforms recovered after the conclusion of stage III and none of the samples which had been exposed to 20 or 40°F were positive for the group. Only one sample was coliform positive at the end of the entire schedule. That was the one which was held at 10°F for the entire 10-day stage II schedule, at 10°F during the 14-day stage IV phase, and at 20°F for the 7-day period simulating the milder of the two stage V schedules.

Regarding the significance of coliforms in the product it has been shown by Wolford (1956) that the presence of small numbers of these organisms is not proof that there has been contamination during production of the product. Vaughn and Murdock (1956) concluded that the presence of these bacteria in frozen orange juice may have no sanitary significance.

As is common with frozen fruit samples, there was variation from sample to sample. Although the trend indicated microbial destruction with prolonged storage, with most severe action when the temperatures were highest, it was found that a study of average counts was necessary to obtain a clear picture of the changes. Apparently, more than 4 days of holding at temperatures of 20°F is necessary to exert a definite effect upon numbers of viable cells in the product. Little difference was noted in plate counts of samples within each lot immediately upon completion of the stage II cycle or after 3 or 6 months of stage III. The average survival in percentage, is shown in table 1.

From this table it can be seen that there was about the same percentage reduction over the preceding stage during the 21 days of stages IV and V as occurred during 180 days at 0°F in stage IV. This indicates that both

**TABLE 1**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Elapsed Time</th>
<th>Survivals</th>
<th>Reduction over Preceding Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Months)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>1.3</td>
<td>43</td>
<td>% 57*</td>
</tr>
<tr>
<td>III</td>
<td>4.3</td>
<td>26</td>
<td>% 70†</td>
</tr>
<tr>
<td>IV</td>
<td>7.8</td>
<td>9</td>
<td>% 25</td>
</tr>
<tr>
<td>V</td>
<td>8.0</td>
<td>4</td>
<td>% 54</td>
</tr>
<tr>
<td>−10°F control</td>
<td>9.0</td>
<td>36</td>
<td>% 64</td>
</tr>
<tr>
<td>−80°F control</td>
<td>9.0</td>
<td>86</td>
<td>% 14</td>
</tr>
</tbody>
</table>

* No analysis run between stages I and II. Reduction is that over initial count.
† Percentage reduction during 6 months (stage III).

**TABLE 2**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Temperature Schedule</th>
<th>Time at Holding Temperature</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(Days)</td>
<td>%</td>
</tr>
<tr>
<td>IV</td>
<td>10°F</td>
<td>14</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>14</td>
<td>6.0</td>
</tr>
<tr>
<td>V</td>
<td>10-20°F</td>
<td>7</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>10-40°F</td>
<td>7</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>20-20°F</td>
<td>7</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>20-40°F</td>
<td>7</td>
<td>2.6</td>
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<tr>
<td></td>
<td>10, 60-20°F</td>
<td>7</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>10, 60-40°F</td>
<td>7</td>
<td>2.7</td>
</tr>
</tbody>
</table>

* Temperature schedules are described in text.
† First figure for stage IV schedule, second figure for stage V schedule.
time and temperature conditions affect the survival of microorganisms in frozen orange concentrate. A more detailed look into the effect of temperature patterns upon survival of microorganisms in the product is supplied in Table 2.

It is of interest that a 12-hr thawing to 60°F followed by refreezing to 10°F (stage II (b)) resulted in less change in survival of bacteria than did a prolonged exposure to 20°F during stage IV. The lethal effect of exposure to elevated temperature is best illustrated by the fact that the lowest survival occurred in samples which were exposed to the most severe temperature conditions.

The plate count of the small lot stored at a constant 40°F temperature dropped to 30 per cent of the initial count in 8 days, 17 per cent in 21 days, 8 per cent in 42 days, 6 per cent in 98 days, 4 per cent in 151 days, and 3 per cent in 187 days. The "Leuconostoc-type" colonies tended to disappear during the storage and yeasts like colonies predominated towards the end of the period.

The gummy "Leuconostoc-type" colonies in all lots studied made up 2 to 10 per cent of all colonies on the plates of the first samples plated. After the product had been exposed to the more adverse temperature conditions, the yeast formers were seldom encountered. Whether these bacteria had been eliminated or were undetected because of low counts can not be stated for certain.

Growth of psychrophilic organisms was not detected in any of the samples, including those exposed to 40°F. Apparently failure of such organisms to grow was related to the pH of the concentrate, which was slightly below 3.5.

If the only factor in frozen-food quality were its microbial content, it might be inferred that refrigerated temperatures above zero benefit the product. Unfortunately, other changes in orange concentrate take place. One, loss of cloud, is a much more sensitive test than changes in bacterial numbers, and is one which more readily reveals degree of change due to time and temperature conditions. Unless the previous history of the sample is known, plate counts made on a single sample of orange concentrate will give but limited information on what has happened to the sample since it was produced. In the study by McColloch et al. (1957), which covered chemical and organoleptic changes in the same lots of concentrate as were studied in work covered by the present paper, it was found that cloud loss occurred in most of the samples and that early exposure to elevated temperatures predisposed the product to greater changes in later phases of its storage life. Flavor changes did not occur until after stage IV was completed. Changes in flavor occurred in samples exposed to the most unfavorable storage temperatures and were most likely to happen in samples having the same storage histories as those samples in which the greatest microbial destruction was found.

Plate counts of frozen concentrated orange juice should not be used as the sole criterion of product quality, as low counts in some cases may be due to exposure to adverse time and temperature conditions which have had adverse effects on the quality of orange concentrate.

**Summary**

Samples of orange juice concentrate were submitted to time-temperature conditions that frozen concentrated orange juice might encounter during its commercial experience. Exposure patterns were evolved and several lots of concentrate were stored under mild to severe conditions simulating these patterns.

Plate counts were found to decrease with time. The most rapid decreases were in samples subjected to the most severe temperature conditions—14 days at 20°F, followed by 7 days at 40°F. Average counts under this schedule showed only 2.6 per cent survival. Concentrate stored continuously in Dry Ice lost only about 14 per cent of its initial population and at −10°F, 64 per cent reduction was found after 9 months.

Coliforms were found in four of five lots at the beginning of the experiment, but were practically eliminated after storage at simulated distribution patterns.

**REFERENCES**


