bate again failed to control *L. dextranicum* adequately at pH 4.5, and growth of this organism was slow in the presence of benzoate alone, whereas the combination prevented growth of all species.

Thus, the results suggest that it may be possible to control microbial growth in chilled citrus salads with a lower level of a 1:1 mixture of benzoate and sorbate than by either alone. It is clear, also, that smaller amounts of preservative are required for effective control at the lower pH values.

## SUMMARY

The preservative action of benzoate, sorbate, and dehydroacetic acid and 1:1 mixtures of pairs of these was measured in a simulated citrus salad cover sirup at 10 C over the pH range 3.5-4.5. Organisms known to cause spoilage in citrus products and used in this study were *Lactobacillus brevis, L. plantarum* var. *mobilis, Leuconostoc dextranicum, L. mesenteroides,* and two unidentified yeasts. All preservatives decreased growth rates or increased death dates. The lactobacilli were controlled by temperature alone, and the leuconostocs by pH alone. Sorbate or dehydroacetic acid was very effective against the yeasts. Benzoate plus sorbate was more effective than either alone.

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# MICROBIAL POPULATIONS OF FROZEN ORANGES

E. C. HILL

*Florida Citrus Commission*

and

F. W. WENZEL

*Florida Citrus Experiment Station*

Lake Alfred

Severe freezes occur in the citrus belt of Florida infrequently, but when they do there is always apprehension among growers and processors about *en masse* fermentation or spoilage of oranges on the trees. Faville and Hill (1) isolated six groups of bacteria from frozen orange concentrate and reported the ability of these microorganisms to grow in filtered orange juice. Hill and Faville (3) inoculated several species of these bacteria into oranges growing on trees. Many of these oranges remained on the trees up to five weeks, permitting the growth of large numbers of the inoculated bacteria with little or no external evidence of internal deterioration. The growth of the bacteria was contingent upon the inoculum reaching the juice sacs of the orange. Samish, Etinger-Tulczynska and Bick (4) examined tomatoes, peas, beans, cucumbers, citrus fruits, grapes, melons, olives, peaches and bananas and concluded that different bacterial species are often present within the tissues of fruits and vegetables, but also stated that citrus fruits rarely contained bacteria.

The purpose of this investigation was to determine the likelihood of widespread microbial deterioration of fruit remaining on the trees after a severe freeze and compare such results with those for fruit which had dropped from the trees after freezing and remained on the ground for varying periods of time.

## EXPERIMENTAL PROCEDURE

During the 1960-61, 1961-62 and 1962-63 seasons, orange trees and fruit were frozen for

1Cooperative research by the Florida Citrus Commission and Florida Citrus Experiment Station. Florida Agricultural Experiment Stations Journal Series No. 1753.

microbial studies, using a mechanical freezing unit which enclosed the entire tree (2). The trees were frozen with varying degrees of severity. Thermocouples inside oranges on the tree indicated the temperature, and fruit was cut to determine the formation of ice crystals. The readings cited here are minimum significant temperatures; however, lower temperatures were sometimes reached for brief periods because of super cooling. The length of time required to reduce the temperature to 32° F. or below was generally a much longer period than the holding time at the minimum temperature.

Generally 20 oranges were used on each sampling date. Oranges which had dropped from the tree were selected at random, avoiding fruits which had obvious indications of decay. The fruit were hand washed with detergent. Just prior to cutting, each orange was minutely examined for general condition and defects and placed in a 1:500 mercuric chloride bath for 5 to 10 minutes. The oranges, after removal from the germicidal bath, were carefully rinsed with sterile water. The fruit was cut with a knife sterilized by heat and the juice extracted by a Juice-O-Mat hand juicer. This extractor was selected because it was constructed in a manner which permitted the removal of all parts which came in contact with the juice, thereby making possible sterilization by heat after each use. The juices, aseptically extracted, were plated individually on orange serum agar, pH 5.5, and incubated 48 hours at 30° C.

Pilot plant runs using freeze damaged fruit were made during several seasons and extracted juices were usually plated on orange serum agar, pH 5.5, and on destrose agar, pH 7.0, and then incubated at 30°C. for 48 hours.

## Results and Discussion

*Fruit on the tree.*—During the 1960-61 season, a Pineapple orange tree was artificially frozen at 22° F. for 8 hours. The oranges from this tree were examined 8 times over a 39 day period and 1 orange had an insignificant plate count of 1,400 colonies per ml., while the other 154 oranges were found free of microbial infestation. Fruit from a Valencia tree held at 20°F. for 4 hours was examined 10 times over a 66 day period and none of the 194 oranges were found to be infested with microorganisms.

Two Parson Brown trees were held at 23°F. for 8 hours during the 1961-62 season. Such rapid dropping of fruit occurred in this instance that only enough fruit remained on the trees for two samplings, 14 and 20 days after treatment. The aseptically extracted juices from 40 Parson Brown oranges were free from microorganisms with the exception of 1 orange which had a very low count of 2,400 colonies per ml. of juice.

A Pineapple orange tree was artificially frozen at 24° F. for 8½ hours, and fruit was examined 12 times over a period of 120 days. The juices from the 240 oranges were free from microorganisms with one exception, and this juice, examined 99 days after freezing, had a plate count of 2,610,000.

Fruit from a Valencia orange tree, which had been held at 23° F. for 11 hours, was picked 12 times during 105 days. One of the 240 oranges examined had a plate count of 5,800 colonies per ml. 56 days after freezing and the juice from another orange had a plate count of 84,000 after remaining on the tree 98 days.

In the 1962-63 season all of the oranges picked were naturally frozen during the severe December cold spell. Hamlin oranges, which had developed "wet peel" ½ to 2 inches in diameter, generally at the stylar area, were examined. The juices from 40 of these naturally frozen oranges were individually plated, 20 shortly after picking and 20 after 6 days storage at 80° F. All of them were found to be free of yeast and bacteria.

A total of 140 Pineapple and 180 Valencia oranges, which had been naturally frozen in December and remained on the trees, were examined periodically throughout the season. Fruit from 2 Pineapple trees was examined 7 times between the 5th and 11th week after freezing. Two oranges had insignificant plate counts of 2,500 and 1,200, while the juice of one orange had 71,000 colonies per ml. Frozen Valencia oranges were picked from three trees and examined 9 times from the 9th to the 19th week after the December freeze. Plate counts for 4 of the juices were 10,000, 3,600, 6,900 and 19,500 colonies per ml.; the remaining juices were free from microorganisms.

Ten Parson Brown and 10 Pineapple oranges, picked at random from several trees, had remained on the trees for approximately 4 months after the December freeze. They had a very poor external appearance, being withered, dry and spongy. When examined these fruit were found to be sterile or to have plate counts of no consequence. Occasionally, an orange which is obviously damaged will remain on a tree. Such a

Valencia orange which was shriveled and had small holes from which insects were entering and emerging, but which nevertheless had remained on the tree 4 months after the December freeze, was picked and the juice plated. The interior of this orange contained large numbers of insect larvae, and the juice had a plate count of 147,000,000 colonies per ml.

During the 3 seasons, in which this study was made, 1249 oranges were examined which were artificially or naturally frozen and allowed to remain on the trees. Only 1 orange contained juice with a plate count of over 1 million colonies per ml. and only 2 juices had counts over 50,000. From the data obtained the conclusion was reached that frozen oranges, picked from trees, were safe to use from a microbiological standpoint; also, that fermentation does not occur in frozen fruit to any practical extent, regardless of how long the fruit remains on the tree after freezing.

Fruit damaged by the freeze of December 1962 was picked immediately after the freeze and throughout the remainder of the season from Parson Brown, Pineapple and Valencia orange trees. Six trees of each variety were used. Over 1,000 oranges were examined and tasted. Although there was a loss of both flavor and acidity in the juice of the Parson Brown and Pineapple oranges one week after the fruit was frozen, no off-flavor or fermentation was detectable by taste even in fruit that had remained on the tree for 3 months. The same was found to be true in fruit picked and tasted following the freezes of the 1957-58 season, as well as those from trees artificially frozen during the 1960-61 and 1961-62 seasons.

*Fruit on the ground.*—After a freeze most of the mature, damaged fruit will generally drop from the tree in about two to three weeks. During the harvesting of the remaining fruit there is some likelihood of dropped fruit, still firm and of good appearance, being included. The portable freezer was used to freeze several trees in order to study the fruit which dropped and remained on the ground. As the oranges dropped they were gathered daily and arranged in groups under other trees from which all fruit had been harvested. Oranges selected for plating were free from obvious microbial infestation, punctures, cracked peel or decay. The juice of the dropped fruit was aseptically extracted and plated using the same technique as that used for fruit picked from trees.

During the 1960-61 season drops were collected from under 3 Pineapple and 2 Valencia orange trees after freezing in the portable freeze chamber. One Pineapple tree was held at 24° F. for 4 hours while 2 other trees were frozen at 20° F. for 4 hours. The juices from 144 oranges, which had remained on the ground from 2 to 22 days, were plated, and 9 were found to contain microorganisms (Table 1) ranging from 13,000 to 416,000,000 per ml. Valencia trees were not frozen for microbiological purposes and, therefore, the length of time the dropped oranges remained on the ground was not known. However, 20 dropped oranges were gathered from beneath a tree which had been held at 20° F. for 4 hours, 68 days previously, and 20 which had dropped from another tree during a 34 day period after being held at 20° F. for 3 hours. The Valencia juice from 1 orange had a plate count of 610,000 per ml. of juice while the others from these

Table 1. Microbiological plate counts on juices from individual, dropped, frozen oranges - 1960-61 season

| Variety | Tree number | Date frozen | Date dropped | Date plated | Days on tree | Days on ground | Total number plated | Number contaminated | Plate count per ml. of juice* |
|---|---|---|---|---|---|---|---|---|---|
| Pineapple | 10 | 1/ 6/61 | 2/3/61 | 2/17/61 | 28 | 14 | 20 | 5 | 13,000 |
| | | " | " | " | " | " | - | - | 416,000,000 |
| | | " | " | " | " | " | - | - | 400,000,000 |
| | | " | " | " | " | " | - | - | 410,000,000 |
| | | " | " | " | " | " | - | - | 6,570,000 |
| Pineapple | 8 | 1/25/61 | 2/1/61 | 2/ 3/61 | 7 | 2 | 20 | 0 | - - - - |
| | | " | " | 2/ 8/61 | 7 | 7 | 20 | 1 | 34,500 |
| | | " | " | 2/15/61 | 7 | 14 | 20 | 0 | - - - - |
| | | " | " | 2/20/61 | 7 | 19 | 20 | 1 | 315,000,000 |
| Pineapple | 5 | 2/ 1/61 | 2/8/61 | 2/28/61 | 7 | 20 | 20 | 1 | 70,000,000 |
| | | " | 2/8/61 | 3/ 2/61 | 7 | 22 | 24 | 1 | 380,000 |
| Valencia | 4 | 2/17/61 | Not known | 4/26/61 | - | - | 20 | 1 | 610,000 |
| | 7 | 3/16/61 | " " | 4/19/61 | - | - | 20 | 0 | - - - - |

* Plated on orange serum agar, pH 5.5.

trees were free from microorganisms (Table 1).

During the 1961-62 season a Parson Brown, a Pineapple and a Valencia tree were subjected to varying degrees of artificially induced cold to cause the fruit to drop. The dropped fruit was treated in the same manner as that during the previous season. The Parson Brown tree was held at 23° F. for 8 hours. The fruit which dropped was examined 7 times after being on the ground from 8 to 23 days. Of the 140 oranges plated, 20 were found to have plate counts ranging from 33,000 to 355,000,000 colonies per ml. The juice from dropped oranges from the Pineapple tree, held 1½ hours at 20° F., was examined 6 times after the fruit had remained on the ground 6 to 31 days. Two of the 120 oranges examined were highly contaminated and 2 had minor infestation. The Valencia tree was held at 22° F. for 4 hours and the fruit which dropped was examined 7 times. The oranges remained on the ground 13 to 28 days with only 1 orange, of the 140 examined, being infested with microorganisms. Table 2 shows the results for the 1961-62 season.

In the 1962-63 season 1 tree was frozen artificially 1 week before the severe natural December freeze. This Pineapple tree was subjected to the extreme conditions of 25° F. for 32 hours. Table 3 shows the results of the plating of 100 oranges from this tree remaining on the ground 21 to 43 days. Twenty-two per cent of the oranges had significant plate counts ranging from 31,000 to a high of 462,000,000 per ml. of juice.

Rapid deterioration of a very large proportion of the dropped fruit occurred because of growth of stem end rot and surface molds and infestation by insects. Because of this and from the data presented, it is evident that microbial contamination was much more frequent and greater in dropped frozen fruit than in that picked from trees, even though the dropped fruit for plating was selected so as to be in relatively fair condition.

While examining the fruit in preparation for extracting the juice, it was observed that many of the oranges had minute holes in the peel which could only be demonstrated by exerting pressure on the fruit under water. The holes

Table 2. Microbiological plate counts on juices from individual, dropped, frozen oranges - 1961-62 season

| Variety | Tree number | Date frozen | Date dropped | Date plated | Days on tree | Days on ground | Total number plated | Number contaminated | Plate count per ml. of juice* |
|---|---|---|---|---|---|---|---|---|---|
| Parson Brown | 7 | 12/19/61 | 12/28/61 | 1/ 5/62 | 9 | 8 | 20 | 0 | - - - - |
| | | " | 12/29/61 | 1/11/62 | 10 | 13 | 20 | 1 | 1,650,000 |
| | | " | 12/30/61 | 1/16/62 | 11 | 17 | 20 | 4 | 131,000,000 |
| | | " | " | " | " | " | - | - | 175,000,000 |
| | | " | " | " | " | " | - | - | 4,600,000** |
| | | " | " | " | " | " | - | - | 335,000,000 |
| | | " | 12/31/61 | 1/17/62 | 12 | 17 | 20 | 5 | 1,170,000** |
| | | " | " | " | " | " | - | - | 72,000,000 |
| | | " | " | " | " | " | - | - | 355,000,000** |
| | | " | " | " | " | " | - | - | 64,000,000 |
| | | " | " | " | " | " | - | - | 33,000** |
| | | " | 1/ 1/62 | 1/19/62 | 13 | 18 | 20 | 2 | 92,000,000 |
| | | " | " | " | " | " | - | - | 135,000 |
| | | " | " | 1/24/62 | 13 | 23 | 20 | 6 | 320,000,000** |
| | | " | " | " | " | " | - | - | 7,300,000** |
| | | " | " | " | " | " | - | - | 135,000,000 |
| | | " | " | " | " | " | - | - | 40,000,000** |
| | | " | " | " | " | " | - | - | 12,000,000** |
| | | " | " | " | " | " | - | - | 430,000** |
| | | " | 1/ 6/62 | 1/25/62 | 18 | 19 | 20 | 2 | 12,000,000 |
| | | " | " | " | " | " | - | - | 158,000,000** |
| Pineapple | 10 | 1/26/62 | 1/31/62 | 2/ 6/62 | 5 | 6 | 20 | 0 | - - - - |
| | | " | 2/ 7/62 | 2/14/62 | 12 | 7 | 20 | 0 | - - - - |
| | | " | 2/ 1/62 | 2/21/62 | 6 | 20 | 20 | 0 | - - - - |
| | | " | 2/ 2/62 | 2/27/62 | 7 | 25 | 20 | 2 | 212,000,000 |
| | | " | " | " | " | " | - | - | 57,000,000 |
| | | " | 2/ 3/62 | 2/28/62 | 8 | 25 | 20 | 1 | 41,500 |
| | | " | 2/ 6/62 | 3/ 9/62 | 11 | 31 | 20 | 1 | 13,000 |
| Valencia | 6 | 2/15/62 | 2/21/62 | 3/ 6/62 | 6 | 13 | 20 | 0 | - - - - |
| | | " | 2/22/62 | 3/15/62 | 7 | 21 | 20 | 0 | - - - - |
| | | " | 2/21/62 | 3/20/62 | 6 | 27 | 20 | 0 | - - - - |
| | | " | 3/ 6/62 | 3/29/62 | 19 | 23 | 20 | 0 | - - - - |
| | | " | 3/ 7/62 | 4/ 4/62 | 20 | 28 | 20 | 0 | - - - - |
| | | " | 3/17/62 | 4/12/62 | 30 | 26 | 20 | 1 | 5,500,000 |
| | | " | 3/26/62 | 4/19/62 | 39 | 24 | 20 | 0 | - - - - |

* Plated on orange serum agar, pH 5.5. ** Micro-hole detected in peel of fruit.

Table 3. Microbiological plate counts on juices from individual, dropped, frozen oranges - 1962-63 season

| Variety | Tree number | Date frozen | Date dropped | Date plated | Days on tree | Days on ground | Total number plated | Number contaminated | Plate count per ml. of juice* |
|---|---|---|---|---|---|---|---|---|---|
| Pineapple | 8 | 12/ 6/62 | 12/13/62 | 1/ 3/63 | 6 | 21 | 20 | 2 | 67,500 |
| | | and | " | " | " | - | - | - | 40,000 |
| | | 12/ 7/62 | 12/13/62 | 1/10/63 | 6 | 28 | 20 | 1 | 1,200,000 |
| | | " | 12/25/62 | 1/22/63 | 18 | 28 | 20 | 4 | 160,000** |
| | | " | " | " | " | " | - | - | 292,000,000** |
| | | " | " | " | " | " | - | - | 10,500,000** |
| | | " | " | " | " | " | - | - | 368,000,000** |
| | | " | 12/25/62 | 1/24/63 | 18 | 30 | 20 | 11 | 106,000,000 |
| | | " | " | " | " | " | - | - | 2,800,000 |
| | | " | " | " | " | " | - | - | 37,000,000 |
| | | " | " | " | " | " | - | - | 284,000,000** |
| | | " | " | " | " | " | - | - | 49,000 |
| | | " | " | " | " | " | - | - | 462,000,000** |
| | | " | " | " | " | " | - | - | 282,000,000 |
| | | " | " | " | " | " | - | - | 269,000,000 |
| | | " | " | " | " | " | - | - | 79,500,000** |
| | | " | " | " | " | " | - | - | 100,000** |
| | | " | " | " | " | " | - | - | 20,000,000 |
| | | " | 12/18/62 | 1/30/63 | 11 | 43 | 10 | 0 | - - - - |
| | | " | 12/21/62 | 1/30/63 | 14 | 40 | 10 | 4 | 61,000,000** |
| | | " | " | " | " | " | - | - | 1,000,000 |
| | | " | " | " | " | " | - | - | 31,000 |
| | | " | " | " | " | " | - | - | 119,000,000 |

* Plated on orange serum agar, pH 5.5. ** Micro-hole detected in peel of fruit.

would then emit a stream of bubbles sometimes smaller in diameter than that of a fine human hair. Note in Figure 1, the bubbles rising on the left side of the immersed orange. The micro-holes were not noticed until the 1961-62 season when they were found to be present in 35% of the dropped Parson Brown oranges examined and in 50% of those fruits found to be infested with microorganisms. Dropped Pineapple oranges in the 1962-63 season were found to have micro-holes in 33% of the fruit examined and in 41% of the oranges infested with microorganisms. On the other hand, of the total number of oranges of all varieties picked from the trees for the 2 seasons, only 6 were found with micro-holes. Many more of the fruit examined probably had micro-holes which went undetected because the oranges had to be pressed very close to the hole for bubbles to be emitted. When an orange was submerged in water and placed in vacuum, small bubbles slowly formed over the entire surface, but if a micro-hole was present a large stream of bubbles burst forth. The presence of these micro-holes could explain the method of entry of microorganisms into apparently sound fruit. The holes would have to penetrate the juice sacs since if they went only as far as the albedo, most bacteria would not multiply under normal conditions (3). The means by which these openings occurred are not known.

During the 1957-58, 1960-61, 1961-62 and 1962-63 seasons, single-strength juices, extracted in the pilot plant from artificially or naturally frozen oranges, were checked for microbial content by plating on orange serum agar and dextrose agar. Using orange serum agar, 114 juices were found to average 110,000 with a range of 2,000 to 1,850,000 colonies per ml. One hundred ten of these juices plated on dextrose agar were found to range from 1,000 to 2,620,000 and averaged 144,000 per ml.

Control batches of juice from unfrozen oranges, plated on orange serum agar, had an average plate count of 51,000 and a range from 1,600 to 250,000 colonies per ml. for 27 pilot plant runs. Using dextrose agar, 25 batches of control juice averaged 69,000 and the range in plate count was from 10,000 to 246,000.

Juice from dropped frozen oranges, extracted in the pilot plant and plated on dextrose agar, averaged 2,450,000 colonies per ml. with a range from 20,000 to 7,930,000 for 20 lots examined. Orange serum agar counts on these same juices ranged from 16,000 to 7,800,000 with an average of 2,570,000 colonies per ml.

## SUMMARY

The juices of individual oranges frozen on trees, both artificially and naturally, over a period of three seasons were plated on orange serum agar to determine the michobial population of each orange. Fruit picked from the trees and dropped fruit from the ground were ex-

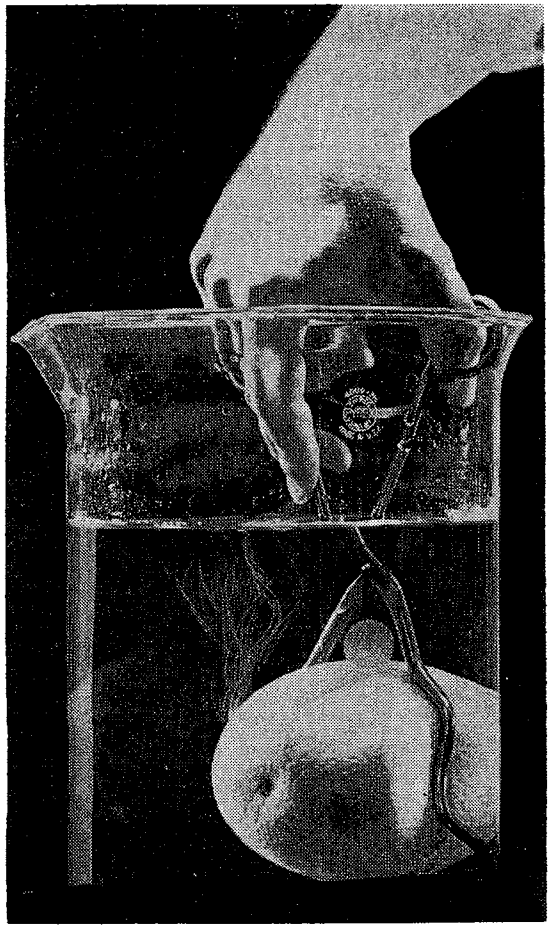

**Figure 1.—Minute bubbles escaping through micro-hole in peel of orange.**

amined. Valencia, Pineapple, Hamlin and Parson Brown oranges picked from 14 to 133 days after freezing showed one orange highly contaminated, two of moderate infestation and 3 with low counts of some consequence from the 1249 oranges examined. Fermentation is of no importance, from a practical standpoint, in frozen fruit as long as it remains on the tree. Microbiologically, frozen fruit picked from trees is safe to use as fresh fruit or for processing.

Frozen fruit deteriorated very rapidly after falling to the ground because of the *growth of* stem end rot and surface molds and infestation by insects. Plate counts in juice from dropped, frozen oranges of fair condition were more frequent and greater than in that from fruit picked from trees. These individual oranges had remained on the ground from 2 to 43 days, and only oranges free from obvious damage were selected. A total of 684 dropped oranges were examined, and 57 were found to be infested with microorganisms, 42 of which had counts of over one million. The range was 13,000 to 462,000,000 per ml.

The occurrence of minute streams of bubbles emitted through micro-holes was noted when some oranges were submerged in water and pressed. These tiny holes, invisible to the unaided eye, could be the means by which microorganisms gain entrance to the interior of the fruit. Not discovered until the 1961-62 season, they were observed in 105 dropped oranges but in only 6 fruit which had been picked from trees.

Naturally or artificially frozen oranges were extracted in a pilot plant during several seasons and the juices plated on orange serum agar. Twenty-seven batches of juice from unfrozen fruit, 114 batches from frozen oranges, and 20 batches from dropped frozen fruit ranged in plate counts from 1,600 to 250,000, from 2,000 to 1,850,000, and from 16,000 to 7,800,000 colonies per ml. of juice, respectively.

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