examined to determine if such organisms were present in this type of fruit. The oranges were sampled after the germicidal rinse and were handled in the same manner as in previous tests. Both the fruit surfaces and the extracted juice of splits and deteriorated fruit were heavily contaminated with microorganisms as indicated in the data presented in Table I. Only catalase positive colonies were noted on the plates made from sound fruit, drops and deteriorated fruit. The split oranges, however, contained both catalase positive and catalase negative gum-forming organisms. Of nine cultures examined from this source, three were catalase positive and six were catalase negative. Three of the catalase negative organisms grew in orange juice producing a buttermilk odor and flavor. Some of the physiological characteristics of the gum-forming organisms that were isolated are presented in Table II. In those cases where the catalase positive organisms grew in orange juice, a bitter flavor was produced, while catalase negative organisms which were V.P. positive developed a characteristic buttermilk odor and flavor. It is planned to study the organisms further in order to identify them.

TABLE I

MICROFLORA ON THE SURFACES AND IN THE EXTRACTED JUICE OF SOUND AND DEFECTIVE FRUIT

CONTAMINATION ON FRUIT SURFACES

(Results Expressed as The Number of Organisms Per Orange)

TYPE OF FRUIT	TOTAL MICROFLORA Test 1	TOTAL MICROFLORA Test 2	GUM-FORMING ORGANISMS Test 1	GUM-FORMING ORGANISMS Test 2
Sound Fruit		74,000		38,000
Drops	64,000	855,000	2,000	371,000
Splits	113,700,000	21,400,000	5,650,000	743,000
Deteriorated Fruit	3,250,000	44,200,000	450,000	24,000

CONTAMINATION OF EXTRACTED JUICE

(Results Expressed as The Number of Organisms Per ml)

Sound Fruit		500		0
Drops	29,000	1,500	7,200	100
Splits	2,800,000	620,000	2,500,000	210,000
Deteriorated Fruit	9,200,000	3,100,000	2,300,000	90,000

NOTE: Catalase negative gum-forming strains were isolated from the colonies obtained from the split oranges.

TABLE II

PHYSIOLOGICAL CHARACTERISTICS OF GUM-FORMING COLONIES ISOLATED

SOURCE OF CULTURES	NO. CULTURES EXAMINED	CATALASE POSITIVE GROWTH NO. (+)	CATALASE POSITIVE GROWTH IN ORANGE JUICE NO.	V.P. (+) NO.	V.P. (-) NO.	CATALASE NEGATIVE GROWTH (-)	CATALASE NEGATIVE GROWTH IN ORANGE JUICE NO.	V.P. (+) NO.	V.P. (-) NO.
Sound Fruit Surfaces	62	62	0			0			
Unsound Fruit	11	5	2	0	2	6	3	3	0
Extracted Juice and Concentrate	28	7	3	0	3	21	18	14	4

Note: A characteristic buttermilk flavor was noted in orange juice inoculated with organisms which produced a positive V.P. test.

SUMMARY

It is evident from these studies that the presence of gum-forming colonies does not always indicate significant off-flavor producing organisms. The surfaces of sound oranges appear not to be a primary source of off-flavor producing bacteria. Unsound fruit, particularly splits, is a source of significant off-flavor producing organisms. Bacteria from this type of fruit apparently "seed" the juice resulting in a potential spoilage hazard where conditions are optimum for their growth. Therefore, the selection of incoming fruit, careful grading, and efficient washing play an important role in controlling these organisms.

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EFFECT OF CONCENTRATION OF ORANGE JUICE AND TEMPERATURE OF STORAGE ON GROWTH AND SURVIVAL OF MICROORGANISMS

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Microbiological stability of orange concentrates when stored at temperatures above freezing has not been studied extensively. Other food products of high density that have been covered more thoroughly, however, give some idea as to what to expect in orange concentrates.

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Dubois and Kew (4) found that there was a notable reduction in microorganisms in orange concentrate at all temperatures below 25° F. Patrick (12) showed that there was a marked reduction in microorganisms in 42° Brix concentrate held at 42° and 0° F. for 111 days.

Curl, Moore, Wiederhold, and Veldhuis (3) noted swelling of cans of 61.7° Brix orange concentrate due to microorganisms within 5 days at 80° F. while no swelling was observed in cans of lower initial microbial count of 64.1° and 64.6° Brix concentrates within 3 months at this temperature. Even pasteurized samples and samples preserved with benzoate swelled in 9 months at 80° F. from chemical decomposition. Curl (2) observed carbon dioxide gas pressure from chemical decomposition in 6 to 9 months at 80° F. The rate of gas formation became more rapid with increases in concentration and temperature of storage.

Owen (11) gave an appraisal of the maximum density limits at which any of the groups of microorganisms known to occur in blackstrap molasses could induce its deterioration and concluded that this extreme upper limit was in the range of 75° to 80° Brix.

Erickson and Fabian (5) reported that yeasts are more tolerant than bacteria to sugars. Forty-five to 60 percent sugar was required to bring about a preserving action with yeasts, whereas with bacteria only 15 to 50 percent was required.

Faville, Hill, and Parish (6) could not find *Escherichia coli* 15 minutes after 42° Brix concentrate was inoculated at 40° C. Hahn and Appleman (7) found that *E. coli* did not survive for 24 hours at −17.8° C. in orange concentrate. McFarlane (10) demonstrated that 99% of the *E. coli* inoculated into single-strength unsweetened orange juice was destroyed in 24 hours at −17.8° C.

If a concentrate could be produced that would be stable at household refrigerator temperatures or above by increasing the concentration of the juice, its production would be economically advantageous. The savings in costs of storage, containers, transportation, and refrigeration would more than offset the added expense of higher concentration.

Experimental Methods

Four batches of single-strength orange juice were obtained from a commercial concentrate plant. Juices obtained on February 13, 1952, were from mid-season oranges, on March 6 and April 3 from mixtures of mid-season and Valencia oranges, and on April 22 from Valencia oranges only.

Each batch of juice was cooled to 40° F. in a coldwall tank as soon as received, passed to the evaporator as needed, and a series of different concentrations prepared. Concentration was accomplished in a falling-film evaporator at 70° F. Concentrate was withdrawn at 40° Brix and at each 5° increase until it became too viscous to handle in the evaporator. These samples were vacuum sealed in 4-oz. lacquered cans and placed in 0°, 35°, 50°, and 60° F. storage for a maximum of 168 days.

In this discussion a "batch" designates a single uniform tank of orange juice from which a "series" of concentrates were prepared. In a "series" the samples of the same concentration are called "sets."

In the first two series, sets ranging from 40° to 70° Brix were prepared. High viscosity was encountered in the third series and it was necessary to omit the 70° Brix set. In the fourth series viscosity was low and it was possible to prepare a 75° Brix set.

Bacterial counts and visual inspection were conducted at scheduled intervals on every concentration of each series that had survived storage. After reconstitution to single-strength juice and suitable dilution, 1 ml. portions of the juice were plated in duplicate on orange serum (pH 5.8), McCleskey's (pH 6.7), and Sabouraud's (pH 5.8) agars. Sabouraud's and orange serum agars were incubated at 98.6° F. while McCleskey's agar was held at 70° F. Total plate counts were made on all three media. The presence of slime and gum forming organisms was shown on McClesky's agar by the occurrence of typical raised or slimy colonies. When swells occurred, microscopic examinations were made of the spoiled concentrates to determine whether the predominating organisms were yeast or bacteria. Cells and formations typical of Leuconostoc bacteria could also be recognized under the microscope.

Coliform observations were made using formate ricinoleate broth (12) and eosin methylene blue (E.M.B.) agar. Ten ml. of formate ricinoleate broth in a fermentation tube was inoculated with 1 ml. of a 1:10 dilution of reconstituted juice. When acid and gas were formed after 48 hours at 95-98.6° F., E.M.B. agar was streaked and observed for *E. Coli.*

Characteristics of the juices used in preparing the series were as indicated in Table 1.

TABLE 1
Single-strength juice characteristics

	Series Number 1 (Feb. 13)	2 (Mar. 6)	3 (Apr. 3)	4 (Apr. 22)
pH	3.61	3.68	3.62	3.65
Brix	10.8°	12.08°	12.7°	11.76°
Titratable acidity as citric acid	0.72%	0.74%	0.91%	0.85%
Brix-acid ratio	15.0:1	16.3:1	14.2:1	13.8:1
Oil, recoverable	0.041%	0.044%	0.048%	0.044%
Suspended solids	11.5%	12.0%	10.5%	10.0%

The juices were about average in Brix, acid, Brix-acid ratio, pH, and suspended solids. The oil contents given are those found in the fresh juice, however, most of the oil would be removed during concentration. The pH values were remarkably uniform for samples selected at different times, as variations from pH 3.4 to 3.8 are common. Orange juices are well buffered and decrease in pH only 0.2 to 0.3 pH units during concentration from single-strength juice to 65° Brix concentrates.

Results and Discussion

Results obtained during the 168-day storage period are shown in Table 2. At 35° F. swelled cans developed in only 3 sets: two of 40° Brix and one of 45° Brix. At 50° F. swelled cans developed in 16 sets: four each of 40°, 45°, and 50° Brix; two of 55° Brix; and one each of 60° and 65° Brix. At 60° F. swelled cans developed in 21 sets: four each of 40°, 45°, 50°, and 55° Brix; three in 60° Brix; and two in 65° Brix.

TABLE 2
Storage life[1] of orange concentrates

Series No.	Orange juice concentrations—Degrees Brix 40°	45°	50°	55°	60°	65°	70°	75°
	days	days	days	days	days	days	days	days
				35° F. Storage				
1	*[2]	*	*	*	*	*	*	—[3]
2	*	*	*	*	*	*	*	—
3	55	84	*	*	*	*	—	—
4	112	*	*	*	*	*	*	*
				50° F. Storage				
1	16	28	44	*	*	*	*	—
2	14	18	47	53	83	167	*	—
3	14	28	28	84	*	*	—	—
4	10	28	28	*	*	*	*	*
				60° F. Storage				
1	6	12	13	56	*	*	*	—
2	6	8	13	22	78	83	*	—
3	4	6	11	28	48	55	—	—
4	6	7	13	20	45	*	*	*

[1]/Time until swelled cans developed.
[2]/* Completed 168 days storage.
[3]/Not packed.

At 35° F. no cans of concentrates of 50° Brix or higher swelled, but at 50° and 60° F. swells were observed in all concentrations below 70° Brix. No swells were observed in the three sets of 70° Brix or the single 75° Brix set.

The main spoilage organisms were observed to be yeasts with the exception of the 40° Brix set of the second series stored at 50° F., where high counts of slime and gum forming bacteria were also found in swelled cans.

In Table 3 average plate counts of remaining samples of the four series are given on orange serum agar. This medium is used extensively in Florida for estimating microbial counts in orange juice and concentrates. This medium favors the growth of yeast but many bacteria will also grow (9). All counts have been calculated on a 12° Brix basis so they may be compared directly. There was a general trend towards lower counts with higher concentrations and also with increasing time of storage.

High counts are evident where swelled cans developed, especially in the lower juice concentrations. In cases where swells developed in some sets and not in the others, it was necessary to discard the swelled cans. In these cases the tables show high counts followed by lower counts. The number of sets remaining at any particular time can be determined by referring to Table 2. There were no marked differences in the numbers of organisms surviving at different temperatures among samples remaining to the end of the storage period. The counts and trends with Sabouraud's agar were quite similar to those with orange serum agar. Plate counts on McClesky's agar were much the same on orange serum and Sabouraud's agars and showed the same trends ex-

Table 3

Average plate counts on orange serum agar (number per ml. 12° Brix juice equivalent).

Storage time	Orange juice concentrations - Degrees Brix							
	40°	45°	50°	55°	60°	65°	70°	75°
Days	35° F. Storage							
0	15,100	12,400	15,800	11,600	9,200	11,800	5,800	6,000
6	9,700	8,400	8,000	6,100	5,200	5,600	5,300	2,900
14	7,300	5,800	4,800	4,000	3,900	4,400	3,000	2,400
28	4,700	4,600	3,800	3,400	3,100	1,000	2,400	900
56	9,900	24,300	2,400	2,000	2,000	1,900	1,800	400
84	56,000	14,600	1,700	1,000	700	700	700	400
112	23,400	6,000	1,700	1,400	600	600	600	300
140	10,000	2,000	4,700	2,400	600	500	500	500
168	10,500	3,500	4,600	2,100	700	500	800	500
	50° F. Storage							
0	15,100	12,400	15,800	11,600	9,200	11,800	5,800	6,000
6	226,000	12,500	8,000	4,800	3,200	3,500	3,300	1,200
14	590,000	150,000	12,300	3,500	2,500	2,100	2,400	1,200
28		580,000	144,000	2,400	900	900	600	400
56				175,000	800	600	500	400
84				9,200	1,100	600	600	500
112				7,700	6,000	1,300	500	300
140				1,100	2,500	500	500	500
168				1,200	2,500	600	500	400
	60° F. Storage							
0	15,100	12,400	15,800	11,600	9,200	11,800	5,800	6,000
6	3,000,000	1,500,000	118,000	5,300	4,100	2,900	2,300	800
14				19,400	1,700	1,300	1,500	700
28				23,800	9,600	600	600	200
56					15,600	1,100	600	400
84					1,200	700	500	500
112					1,100	800	500	400
140					900	3,000	1,100	300
168					1,100	900	600	400

cept in case of spoilage. With spoilage, higher counts were generally found on McClesky's agar than on the other two.

The colonies formed by slime and gum formers on McClesky's agar were not suited to accurate counts because they tended to be large and frequently ran together. The presence or absence of these organisms was noted, however. Usually the number was small in comparison to the total count. Organisms in this group were of interest because they have been associated with off-flavors in concentrated orange juice (8).

Table 4

Survival of slime and gum forming bacteria.

Storage time	Orange juice concentrations - Degrees Brix						
	40°	45°	50°	55°	60°	65°	70°
Days	35° F. Storage						
0	1,2,3,4 f/	1,2,3,4	1,2,3,4	1,2,3,4	1,2,3,4	1,2,3,4	2, 4
6	1,2,3,4	1,2,3,4	1,2,3,4	1,2,3,4	2,3,4	2,3,	2,
14	1,2,3,4	1,2,3,4	1,2,3,4	2,3,4	2,3,4	2,3,	2,
28	1,2, 4	1,2, 4	1,2,3,4	2,3,4	2,3,4		2,
56	1,2,	1,2,	1,2,3,4,	2,3,4,	2,3,		2,
84	1,2,	1,2,	1,2,	2,	2,		2,
112	2,	1,2,	2,	2,	2,		2,
140	2,	1,2,	2,	2,	2,		2,
168	2,	2,	2,	2,	2,		2,
	50° F. Storage						
0	1,2,3,	1,2,3,4,	1,2,3,4	1,2,3,4,	1,2,3,4	1,2,3,4	1,2, 4
6	1,2,3	1,2,3,4	1,2,3,4	2,3,4	1,2,3,4	1,2,3,	1,2, 4
14		2, 4	2,3,4	1,2,3,4	1,2,3,	1,2,3,	1,2,
28				1,	1,	1,	1,
	60° F. Storage						
0	1,2,3,	1,2,3,	1,2,3,4	1,2,3,	1,2,3,4	1,2,3,4	1,2, 4
6	1, 3,		2,3,4	1,2, 3,	1,2,3,4	1,2,3,	1,2,
14				1,2,	1,2,	1,2,	1,2,
28				1,	1,	1,	1,

f/ The numbers indicate the series in which the organisms were found.

Slime and gum formers were observed in all four series of samples and survived for much longer periods at 35° F. than at higher temperatures as shown in Table 4. Survival was quite general up to 28 days and spotty thereafter, except that in the second series they survived in all concentrations but 65° Brix for the entire storage period. At both 50° and 60° F. survival of slime and gum formers was spotty after 6 days, and none were observed after 28 days.

Coliforms were not recovered from any samples after 14 days of storage. They were recovered from the original juice, from 40° to 65° Brix sets immediately after preparation, from 40° to 50° Brix sets stored at 35° F. for 6 days, and from 40° and 45° Brix sets stored at 50° F. for 6 days. These results indicate that within the ranges investigated, the higher storage temperatures and higher concentrations were unfavorable to survival of coliforms. When acid and gas were observed in inoculated formate ricinoleate broth fermentation tubes, streaks were made on E.M.B. agar to check for the presence of *E. coli*. No *E. coli* type colonies were noted, only the pink colonies of *Aerobacter* type being present.

Gram negative nonspore-forming rods and slow lactose-fermenting yeasts which produced acid but no gas in formate ricinoleate broth were observed sporadically during storage. These acid-forming rods produced gray or blue-gray colonies on E.M.B. agar.

Summary

Four series of concentrated orange juices of 40° to 70° Brix in 5° steps were prepared from Florida mid-season and Valencia oranges. Samples were stored at 35°, 50°, and 60° F. for 168 days and examined periodically for can swelling and microbial content.

No cans of concentrated orange juice of 50° Brix or higher swelled in 35° F. storage, but at 50° and 60° F. swells were observed at all concentrations below 70° Brix. Yeasts were the main spoilage agents.

Plate counts showed a tendency to decrease with higher concentrations of orange juice and with increasing lengths of storage time. McClesky's, Sabouraud's and orange serum agars showed similar average plate counts except in case of spoilage. When this occurred, McClesky's agar generally had higher plate counts.

Slime and gum forming bacteria decreased in number more rapidly at 50° and 60° F. than at 35° F. At the two higher temperatures these organisms were not noted after 28 days while at the lower temperature they continued to be found to the end of the 168-day storage period.

Coliforms were found in 40° through 65° Brix concentrated orange juices when prepared, but after six-day storage they were found only in 40°, 45°, and 50° Brix concentrates at 35° F.; and 40° and 45° Brix concentrates at 50° F. After 14-day storage, no coliforms were found. Coliforms with the appearance of *Aerobacter* types but not of *E. coli* types were noted on E.M.B. plates.

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