SOME OBSERVATIONS OF GUM-FORMING ORGANISMS FOUND ON FRUIT SURFACES

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The two most prevalent types of bacteria known to cause off-flavors in orange concentrate belong to the genera *Lactobacillus* and *Leuconostoc,* Murdock et al. (3), and Hays and Riester (2). These organisms grow rapidly in both single strength juice and 20° Brix concentrate producing flavors which have been described as being similar to "buttermilk". Colony characteristics have been used as a criterion to determine the presence of these acid tolerant off-flavor producing organisms. This is particularly true with the Leuconostoc bacteria which produces a characteristic "gum colony" on several citrus media including Orange Serum Agar, Murdock et al. (4). The presence of these organisms in orange juice and concentrate has been considered a potential spoilage hazard. It has generally been assumed that the principal source of off-flavor producing organisms is the fruit; therefore, the selecting, inspecting, washing, and sterilizing of fruit is of prime importance. Biesel (1) and Wolford and Berry (6) noted that juice extracted from defective fruit was much more greatly contaminated than the juice extracted from normal fruit. Hays and Riester (2) noted that some oranges, which by casual inspection appeared sound, actually contained soft spots. The interior of these oranges was a mass of deteriorated pulp, which was heavily contaminated with microorganisms. The ability of these organisms to grow in orange juice and produce off-flavors was not investigated.

As part of a comprehensive study of fruit surface contamination, it was decided to investigate the incidence and significance of gum-forming bacteria which are easily detected on conventional citrus media, since little or no data are available regarding presence of these organisms on fruit surfaces.

EXPERIMENTAL PROCEDURE

The number of microorganisms on the surfaces of Valencia oranges was determined over a 10-day period under fruit handling operations believed to be typical. The oranges were sampled from the trucks as they were being unloaded and from the various steps in handling the fruit up to the extractors. A slight deviation of the standard rinse test was used to determine the number of microorganisms on fruit surfaces. In this study, a sample consisted of 10 oranges distributed equally in two sterile pails, one of which contained rinse water. The pail with rinse water and oranges (containing a small amount of wetting agent) was placed on a mechanical shaker for three minutes. The rinse water was then transferred to the other pail and it was shaken for a similar period. The final rinse water was then plated in the usual manner. Only sound fruit was sampled. McCleskey's Agar was used as the medium because of its specificity for the growth of gum-forming organisms. Orange Serum Agar was used to determine the total bacterial population on fruit surfaces. The plates were examined after 72 to 96 hours of incubation at 20° C. (68°F.). Representative gum-forming colonies were picked from each plate and streaked on Orange Serum Agar slants for future reference. In counting the McClesky's Agar plates, only the number of gum-forming colonies was recorded. The catalase reaction of each colony was determined according to the procedure suggested by Vaughn (5) which consists 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, they were considered potential off-flavor producing organisms. The Voges-Proskauer (V.P.) test was made by growing the organism in sterile orange juice and adding alpha-naphthol and KOH creatine reagents to the culture. A positive reaction was characterized by an intense rose-pink color which developed in a few minutes.

RESULTS

Hundreds of gum-forming colonies were examined during the course of this study. It was observed that certain gum-forming strains of bacteria produce colony characteristics on McCleskey's Agar and Orange Serum Agar which

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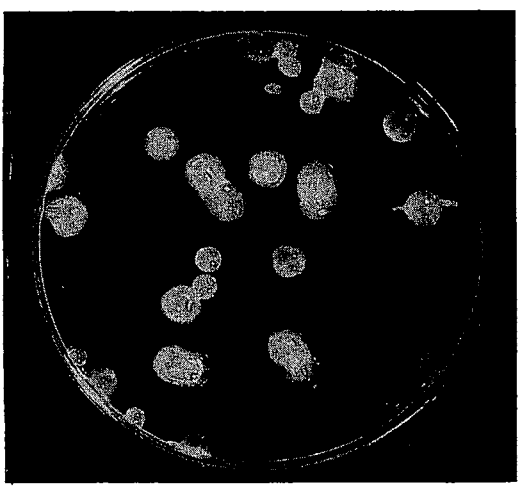
could not be differentiated from off-flavor producing types. True gum-forming organisms belonging to the genus *Leuconostoc* are coccoid in shape and catalase negative. All colonies examined on plates made from rinses from sound oranges were catalase positive. The isolates were coccoid in shape and failed to grow in orange juice. Figure I shows colony

Fig. 1: Serial dilution plate of rinse water from fruit surface studies showing characteristic gum-forming colonies.

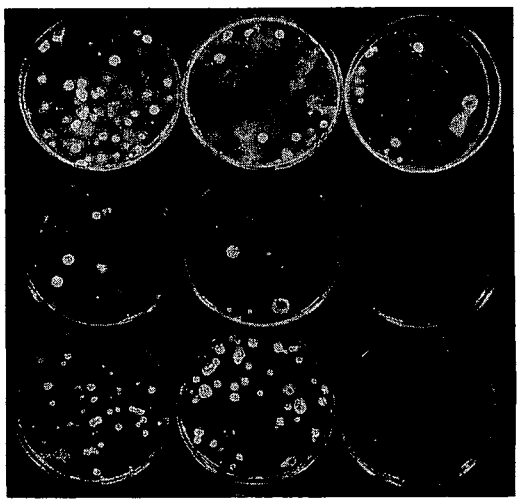


McClesky's Agar Plate after 72 hours of incubation at 20°C. Dilution 1 to 100. Gum-forming colonies catalase positive. Organism failed to grow when inoculated into orange juice.

characteristics of typical gum-forming organisms after 72 hours of incubation at 20° C. This plate represents the microflora found on the fruit surfaces from one sampling station.

Figure II is a photograph showing the colony characteristics of the microflora found on the surface of oranges after various fruit handling operations. The top row of plates contains organisms obtained from unwashed fruit (note mixed flora which were yeasts, molds, and bacteria). The middle set of plates, reading left to right, shows the flora after the initial washing operation and before and after the germicidal rinse. In the same manner, the bottom row of plates depicts contamination before (first two plates) and after (last plate in the lower righthand corner) the final chlorine rinse. The colonies shown were obtained after 96 hours of incubation at 20° C. using a dilution of 1 to 100. The gum-forming organisms in this photograph, as in Figure I, were all catalase positive.

Fig. 2: Serial Dilution Plates showing Microflora on fruit surfaces at various points in fruit handling process.



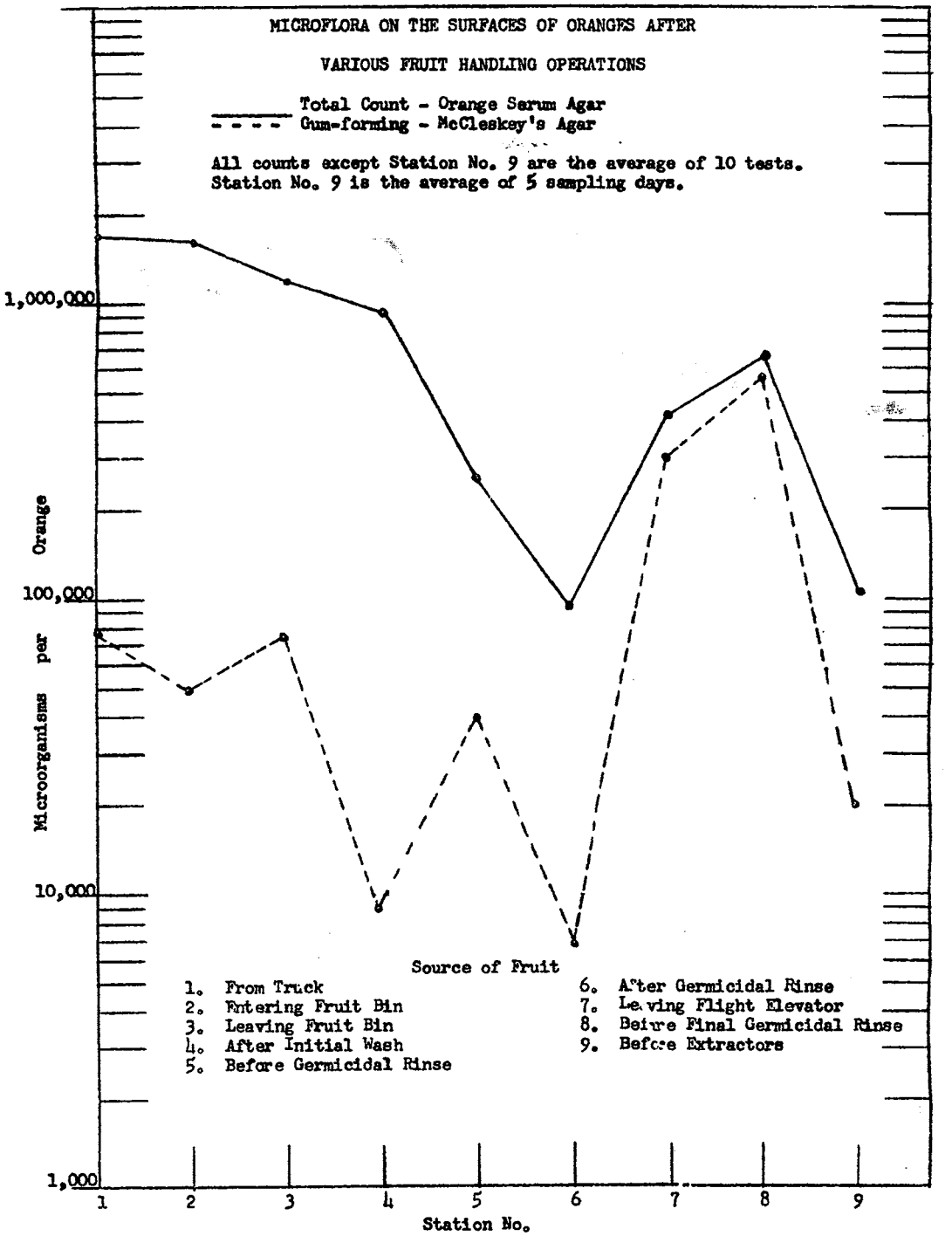
McClesky's Agar Plates after 96 hours of incubation at 20°C. Dilution 1 to 100. Top row, L. to R: From truck, entering fruit bins, leaving fruit bins. Middle row: After initial wash, before germicidal rinse, after germicidal rinse. Bottom row: Leaving flight elevator, before final germicidal rinse, before extractors.

The total microflora and the number of gum-forming organisms found on fruit surfaces after various fruit handling operations are presented in Figure III. All counts except those from station No. 9 are the averages of 10 tests. The count from station No. 9 represents the average count made over 5 days. The data show that the fruit is recontaminated in subsequent fruit handling operations after it leaves station No. 6 (germicidal rinse). The predominant flora on fruit surfaces, as the data show, were gum-forming organisms.

Off-flavor producing, gum-forming microorganisms are known to be present in extracted juice and concentrate. Of 647 colonies examined from plates made from this source, 18% were gum-formers and 82% were of the nongum-forming type. Of the gum-formers, 16% were catalase positive and 2% were catalase negative. The catalase negative organisms grew in orange juice producing a buttermilk odor and flavor.

The presence of significant gum-forming organisms in the finished product, which apparently were not on the surfaces of sound fruit, indicate that these organisms entered the plant from other sources. Defective fruits represented by drops, splits, and deteriorated oranges (soft spots and partially rotten) were

FIGURE III


MICROFLORA ON THE SURFACES OF ORANGES AFTER
VARIOUS FRUIT HANDLING OPERATIONS
Total Count - Orange Serum Agar
Gum-forming - McCleskey's Agar
All counts except Station No. 9 are the average of 10 tests.
Station No. 9 is the average of 5 sampling days.
1,000,000
100,000
10,000
1,000
Microorganisms per Orange
1
2
3
4
5
6
7
8
9
Station No.
Source of Fruit
1. From Truck
2. Entering Fruit Bin
3. Leaving Fruit Bin
4. After Initial Wash
5. Before Germicidal Rinse
6. After Germicidal Rinse
7. Leaving Flight Elevator
8. Before Final Germicidal Rinse
9. Before Extractors

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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