

Survival Times of Selected Enteropathogenic Bacteria in Frozen Passionfruit Nectar Base

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ABSTRACT

AEA, RAYMOND T. F. (University of Hawaii, Honolulu), AND O. A. BUSHNELL. Survival times of selected enteropathogenic bacteria in frozen passionfruit nectar base. *Appl. Microbiol.* **10**: 277-279. 1962.—Five test organisms were used: *Escherichia coli*, *Salmonella typhosa*, *Salmonella schottmuelleri*, *Salmonella enteritidis*, and *Shigella paradysenteriae*. Even when large inocula of these test cultures were introduced into fresh passionfruit nectar base, all test organisms were killed within 1 to 2 hr, provided the nectar base was held at room temperature for more than 1 hr before freezing. If the nectar base was frozen immediately after inoculation, four of the five test organisms were eliminated almost as quickly. But the fifth, *Salmonella enteritidis*, proved to be exceptional: it was being recovered after 90 days of storage at -20°C , when the last available sample was analyzed.

In another paper (Aea and Bushnell, 1962) we have discussed the manner in which passionfruit nectar base is prepared commercially, the composition of passionfruit juice and of the nectar base, and the results of studies upon the normal microflora of the frozen nectar base. Because of our concern that passionfruit nectar base, like other fresh-frozen foodstuffs, might harbor intestinal pathogens, we undertook the investigations reported in the present paper.

MATERIALS AND METHODS

Five strains of gram-negative, aerobic, intestinal bacteria were used: *Escherichia coli*, for the purpose of establishing techniques; and the pathogens *Salmonella typhosa*, *Salmonella schottmuelleri*, *Salmonella enteritidis*, and *Shigella paradysenteriae*. These organisms were obtained from the Bureau of Laboratories, Department of Health, Honolulu.

Each test organism was studied in the following manner. A 1:100 dilution was prepared from an 18-hr culture grown in nutrient broth at 37°C . One milliliter of this 1:100 dilution was added to 200 ml of freshly prepared passionfruit nectar base adjusted to approximately 50% soluble solids. Because we wished to study the product just as it is

¹ The pH was 3.0, and titrable acidity ranged from 2.25 to 2.50%, expressed as citric acid.

provided to the consumer the nectar base was not sterilized or adjusted in pH¹ before the pathogens were added. The test organisms were distributed throughout the nectar base by means of a Waring Blendor, after which 10-ml portions of the mixture were transferred into sterile screw-cap test tubes. Five of these tubes were set aside to be tested for the effect of different intervals of exposure to room temperature before freezing. The rest of the inoculated samples were quick-frozen by immersion in beakers of 95% ethyl alcohol kept in a freezer at -20°C . About 20 min were required to freeze the contents of these tubes. Elapsed time between inoculation of the nectar base and placement of the tubes in the freezer was never longer than 30 min. Portions of the uninoculated nectar base were retained for determinations of pH and titratable acidity, and for plating to determine whether any intestinal gram-negative bacilli were naturally present. The number of test organisms introduced in the inocula was determined by plate counts prepared from the 1:100 dilutions.

These preliminary arrangements were concluded just in time to begin the study of the five inoculated samples which had not been frozen. The temperatures to which these tubes of inoculated nectar base had been subjected are summarized in Table 1.

The specimens which had been frozen immediately were analyzed at intervals of 1 hr for the next 8 hr, then at 24 and at 48 hr after freezing. The contents of the frozen tubes were thawed in a 45°C water bath for 3 to 5 min and the tubes were shaken vigorously before dilutions were made.

Selective media were used in all attempts to recover the test organisms: Violet bile red agar (Difco) for *E. coli*; SS agar (Difco) for *S. paradysenteriae*; and Bismuth sulfite agar (Difco) for the *Salmonella* species. Enrichment cultures, using Tetrathionate broth (Difco), were also prepared in every instance when the *Salmonella* organism was being sought.

The survival time of each test organism was determined in three separate trials.

RESULTS AND DISCUSSION

The pertinent data are summarized in Table 2. They show that passionfruit nectar base was not a hospitable menstruum for the test organisms, even when relatively large numbers of them were introduced into the substrate,

and especially when the nectar base was held at room temperature for more than 1 hr before it was frozen. *S. typhosa* and *S. schottmuelleri* seemed to be slightly more susceptible to the destructive effects of the juice than were the three other organisms; but all five of them were eliminated from the microflora within 2 hr after their introduction when the juice was not frozen at once. Immediate freezing of the nectar base permitted four of the test organisms to survive for only slightly longer periods. But *S. enteritidis* proved to be surprisingly hardy in the quick-frozen mixture: it was still being recovered after 90 days, when the last available sample was analyzed.

As was observed in samples reported in our earlier paper, no gram-negative bacilli of the colon-typhoid-

dysentery groups were found in the normal flora of any of the samples employed in these determinations of survival time. The samples were by no means sterile, however, and showed the usual microflora of fresh nectar base.

These results indicate that if the five enteric organisms (and, presumably, others of their relatives) were to gain entrance to passionfruit nectar base during its production, they would present no hazards to the health of consumers, provided that processors continue their practice of allowing the mixture to stand at room temperature for several hours before freezing it. During that period of exposure, the combined effects of pH, fruit acids, high osmotic concentrations, and, perhaps, other antibacterial substances which might be present in passionfruit juice, are lethal for the intestinal gram-negative aerobic bacilli.

On the other hand, immediate freezing of the nectar base delayed the effects of the bactericidal agents present in it. If some processor should rush his product into the freezer too soon for these agents to exert their effect, he might succeed, ironically, in sparing some pathogens. The ability of *S. enteritidis* to survive for 90 days, and perhaps longer, demonstrates the dismaying resistance of at least this one strain of *Salmonella* to influences which were fatal to other pathogenic intestinal bacilli. Our observations are sustained by the investigations of Berry (1927), who found that this organism survived in frozen butter up to 272 days, and by those of Wallace (1938), who reported a persistence in ice cream for 7 years.

The survival of *S. enteritidis* for such a long time in nectar base frozen within 30 min of inoculation probably is an artifact of experiment rather than a normal occurrence. Nonetheless, the fact that it can persist at all suggests that survival times of other intestinal pathogens should be determined.

In our tests no attempt was made to identify the components present in passionfruit nectar base which were responsible for eliminating the enteropathogens. Workers who have studied other frozen foodstuffs or their constituents (Nunheimer and Fabian, 1940; Erickson and Fabian, 1942; McFarlane, 1942; Faville, Hill, and Parish, 1951; Rushing, Patrick, and Veldhuis, 1954) have shown that pH, organic acids, osmotic concentrations, and low temperatures are involved in reducing the numbers of microorganisms in frozen fruit products. Moreover, according to studies made in this laboratory (Bushnell, Fukuda, and Makinodan, 1950), the fruit juice and extracts of all parts of *Passiflora edulis* forma *flavicarpa*, and of certain other species of *Passiflora* as well, contain substances which are antibiotic to *Staphylococcus aureus*, *E. coli*, and *Pseudomonas aeruginosa*. These substances appear to exert their bactericidal effect independently of pH and osmotic concentration and deserve further investigation. Our studies with the enteric pathogens indicate that these organisms, too, are affected by the antibacterial agents present in passionfruit juice.

TABLE 1. Exposure temperature of inoculated nectar base

Tube no.	Time of exposure	
	Room temperature (25 C)	Freezer (-20 C)
	hr	hr
1	1/2	
2	1	
3	1	1
4	2	
5	2	1

TABLE 2. Survival times of five enteric organisms inoculated in passionfruit nectar base and stored at 25 C and at -20 C.

Test organism	Trial	No. of organisms inoculated per ml of nectar base	Survival times	
			At 25 C	At -20 C
			min	hr
<i>Escherichia coli</i>	1	35,000	60	7
	2	25,000	60	6
	3	24,000	60	6
<i>Shigella paradysenteriae</i>	1	61,500	60	2
	2	20,500	60	1
	3	16,000	60	1
<i>Salmonella typhosa</i>	1	19,000	30	0
	2	33,000	30	0
	3	28,000	30	0
<i>Salmonella schottmuelleri</i>	1	58,000	30	1
	2	46,500	30	1
	3	61,000	30	1
<i>Salmonella enteritidis</i>	1	60,000	60	48*
	2	122,000	60	83†
	3	41,000	60	90†

* Not enough samples were prepared to carry the test beyond 48 hr.

† Not enough samples were prepared to carry the test beyond 83 and 90 days.

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