FACTORS TO CONSIDER CONCERNING THE OCCURRENCE OF COLIFORMS IN CITRUS PRODUCTS

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Since the introduction of frozen concentrated orange juice to consumers, approximately 12 years ago, the question of the sanitary significance of coliform bacteria in this product has been raised from time to time. Also, with the greater distribution of refrigerated citrus products, such as chilled orange juice, by dairies and retail stores, questions are again being asked about the occurrence of coliforms in these products. When one considers the emphasis that has been placed for a long time on the sanitary significance of coliform bacteria in water, dairy and other food products, it is not difficult to understand why inquiries are made when these organisms are found in citrus products.

This paper discusses briefly, on the basis of available data and other information, factors that should be considered by all persons who are concerned about the presence of coliforms in citrus products, so that they may interpret better results obtained from the microbiological examination of these products. Another purpose is to assemble and include all of the references in the literature covering the many investigations about the occurrence, methods of detection, differentiation, sources, survival and significance of coliform bacteria in citrus juices and concentrates. These studies have been made by the citrus industry, the frozen food industry, governmental control and research agencies, container manufacturers and various universities. A careful and thorough study of the results of these investigations indicates that the occurrence of coliform bacteria in frozen citrus concentrates and other processed citrus products is of no sanitary significance and that the health of the public has not been endangered in the past and is not liable to be in the future from the consumption of these products.

DETECTION OF COLIFORMS

The coliform group of organisms as defined in Standard Methods for the Examination of Water and Sewage (3) includes "all aerobic and facultative anaerobic, Gram-negative non-saprophytic bacilli which ferment lactose with gas formation." Since coliform bacteria ferment lactose, various media containing this sugar have been used for their detection and separation from other microorganisms in water, dairy and other food products. The inoculation of a medium with orange juice results in the addition of sucrose, dextrose, levulose and other sugars, which are

1 Cooperative publication by the Florida Citrus Experiment Station and Florida Citrus Commission. Fla. Agr. Exp. Station Journal Series, No. 884.
natural constituents of the juice. If microorganisms other than coliforms, such as yeasts, in the citrus juice can ferment these added sugars, gas will be produced and false positive presumptive tests will result. Such false tests can be prevented to some extent, but not entirely, either by adding to media substances that inhibit the growth of organisms responsible for the false tests or by increasing the temperature of incubation, which favors the growth of coliform bacteria but hinders that of yeasts and other organisms. Various investigators \((8, 12, 24, 27, 44, 45)\) have used and compared different media including lactose, lauryl tryptose and brilliant-green lactose bile broths incubated at \(27^\circ C\); also, Vaughan-Levine boric acid broth at \(43^\circ C\) and modified Eijkman medium at \(46^\circ C\). When these media are inoculated with citrus juices, some false positive presumptive tests may still result even though inhibitors and increased temperatures of incubation are used. Therefore, confirmed and completed tests for coliform bacteria must be made, according to standard methods \((3)\), if the presence of such bacteria is to be definitely established. Also, any attempt to estimate in orange juice coliform group density or compute the most probable number, as in the examination of water \((3)\), may give erroneous and misleading results. This was again pointed out recently by Wolford \((45)\), who stated that if orange juice is tested for its coliform index, all positives should be confirmed by microscopic examination. Martinez and Appleman \((24)\) reported the futility of using eosin methylene blue plates for a confirmed test, since orange juice sometimes contained lactose fermenting yeasts that can give colonies on this medium identical in appearance to Escherichia coli. Violet red bile agar may be used for the detection of coliforms in citrus products, but the isolates must be confirmed by microscopic examination or differential tests.

**Differential of Coliforms**

Biochemical reactions of coliform bacteria found in water, dairy and other food products are used for determining their sanitary significance. The coliform group, according to the American Public Health Association \((3)\), may be classified into the *Escherichia coli*, *Aerobacter aerogenes* and *Escherichia freundii* (or intermediate) types on the basis of the IMVIC reactions. The *E. coli* types are considered to be of fecal source and of sanitary significance, whereas the other types are considered to be of non-fecal origin.

Results from investigations reported in the literature \((28, 31, 34, 43, 46, 47, 48, 49)\) on the differentiation of coliforms isolated from orange juices and concentrates show that most of these organisms have IMVIC reactions which classify them as *Aerobacter* or intermediate types. Those having IMVIC patterns, \(+ + - -\), or \(- + - -\), corresponding to *E. coli* types, are in the minority. For example, Wolford \((43)\) reported that from 1185 initial inoculations into lactose broth, a total of 236 coliform cultures were isolated from 79 samples of frozen orange juice. Forty-six of these cultures resembled *E. coli*, 20 resembled the intermediates and 170 resembled *Aerobacter* types. Similar results have been reported by other investigators \((27, 38)\).

Patrick \((31)\) determined the IMVIC and many other biochemical reactions of 217 coli-like cultures isolated from orange concentrates and damaged oranges. Among these reactions were nitrate reduction and gelatin liquefaction; cellulobiose, salicin, sucrose, starch and inositol fermentations; also utilization of urea and uric acid. Only 2 of the 217 coliform cultures studied were confirmed in all tests to be *E. coli*. Thus, if variations in biochemical reactions are taken into consideration, the generic classification of coliforms isolated from citrus products becomes difficult. Coliform bacteria found in orange juices have also been reported \((22, 24, 34, 43)\) to have some of the characteristics of the genus *Erwinia*. Kauffmann \((21)\) indicated that there are cultures not true to the genus *Escherichia* or to the genus *Klebsiella*, but occupy a position between these two genera. This seems to be the position of many coliform types found in citrus juices, if their biochemical characteristics are taken into consideration.

**Sources of Coliforms**

Many investigations \((15, 19, 26, 27, 28, 29, 30, 34, 36, 46, 47, 48, 49)\) have been concerned with sources of the coliform bacteria found in citrus products. The effect of the condition of fruit has been checked by examination of immature, overmature, soft and damaged fruit, as well as that in good condition. Oranges collected from many places have been examined, including those picked in the grove or obtained from field boxes, trucks, fruit bins and packing houses. Both the exterior and the juice were checked for the occurrence of coliforms. Results of these investigations showed that coliform bacteria can be isolated from the exterior surfaces or juice of oranges collected from the different places; even fruit picked aseptically into sterile containers sometimes yielded coliforms. However, there was no appalling evidence that the coliform bacteria found should cause alarm. The incidence of *E. coli* types from such fruit was low. Rakieten *et al* \((32)\) examined juices that were freshly extracted from oranges in different homes, as well as samples of reconstituted frozen orange concentrates, and reported the occurrence of coliform bacteria in the freshly extracted orange juices, but
none were found in the reconstituted frozen concentrates.

Wolford and Berry (47) and Wolford (48, 49) reported that the physical condition of the oranges used had a decided influence on the plate counts and the numbers of coliform bacteria found in frozen citrus juice. Juice prepared experimentally from “soft rot” Valencia oranges (47) contained 2,500 times as many microorganisms as juice from sound fruit. The incidence of coliform bacteria was higher also. Patrick (30) isolated coliforms from soft and ruptured fruit; he also reported that coliform bacteria were found on fruit infested with scale and that fruit flies could carry coliform contamination. Therefore, fruit exteriors may be a source of coliforms found in juices. However, the methods used today to prepare fruit for extraction are not favorable for such contamination.

The scale infestation and dirt clinging to the peel is removed by a thorough soaking and scrubbing, followed by clear water and chlorine-water rinses. Contamination on the peel due to human contacts, trucks, storage bins and grove-lands is removed also. The thermal stabilization processes used today for the inactivation of enzymes prior to or during concentration of citrus juices, as discussed by Wenzel and Moore (42), also are destructive to many coliform types that may be in these juices.

Good sanitary practices are used today in commercial concentrate plants, as has been indicated by Brokaw (10), Duck (11), and others (7, 39, 40). However, coliform bacteria sometimes are present in citrus concentrates even with all of these good sanitary and clean-up procedures. Therefore, it is not surprising that various investigators (26, 28, 29, 36) have reported, before the use of today’s sanitary practices, that equipment in processing plants was a source of coliforms then found in citrus juices.

**Significance of Coliforms in Citrus Products**

The significance of coliform bacteria in water, milk and other food products, including frozen foods, has been discussed extensively (3, 4, 9, 10, 20). Hunter (20), Levine (23) and others (1, 2, 5, 14, 41) have discussed the uses and limitations of the coliform group in sanitary control of food production. They disapprove the use of coliform organisms as indices of pollution when found on foods without correlative data to establish the route of contamination on that class of food.

Coliform bacteria found in citrus juices and concentrates are far less numerous than that occurring in other food products, such as ice cream, shellfish or nutmeats. Also, since citrus products are acid foods, coliforms die off rapidly when inoculated or introduced into these products at room temperature; however, it has been shown (6, 13, 17, 18, 25, 34, 35) that they survive for longer periods of time as the temperature is decreased and have been known to remain viable for 12 months in orange concentrate stored at 0°F.

Finally, the significant fact should be mentioned that consumers are today using approximately 65 million gallons of frozen orange concentrate yearly, as well as very large quantities of other citrus products, without any apparent ill effect upon their health. Thatcher (37) did not recommend the setting up and using microbial standards as criteria of healthfulness of frozen citrus juices.

Considering these factors, along with the other information discussed briefly in this paper, we must conclude that the occurrence of coliform bacteria in citrus products is of no sanitary significance, an opinion that has also been reached by other persons who have investigated the available information concerning this question.

**Summary**

The consideration of available information from many investigations relative to the occurrence of coliform bacteria in frozen citrus concentrates and other processed citrus products indicates that such organisms are of no sanitary significance. Methods of detection and differentiation, sources, survival and significance of coliforms in citrus products are discussed briefly. Also pointed out is the significant fact that millions of gallons of citrus juices are being consumed annually without any apparent ill effect on the health of consumers.

**Literature Cited**

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