Significance of the Presumptive Coliform Test as Applied to Orange Juice

E. R. Wolford

Fruit and Vegetable Chemistry Laboratory, 1 Pasadena, California

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The presumptive test for the determination of coliforms in food products is based on the inoculation of tubes of lactose broth with specified amounts of the material (A.P.H.A., 1946). Fermentation of the sugar with formation of gas indicates a positive presumptive test for coliforms. This test was originally developed for the examination of water and milk samples, but in recent years it has been used for the examination of many food products regardless of differences in their chemical composition. With the phenomenal development of frozen concentrated orange juice, the presumptive test has been widely employed by regulatory and industry laboratories in the examination of these products for coliforms.

Orange juice is a complex mixture of chemicals including carbohydrates, free amino acids, vitamins, enzymes, and other compounds. For example, approximately 76 per cent or higher of the soluble solids of orange juice is carbohydrate. McCready et al. (1950), using chromatographic techniques, found that most of this carbohydrate is sucrose, dextrose, and levulose in the ratio of 2:1:1. Therefore, an orange juice containing 10 per cent, or more, of these fermentable sugars is not uncommon.

When 10 ml of lactose broth is inoculated with 1 ml of orange juice, two-thirds of the sugars in the inoculated broth may be other than lactose. It is not improbable that gas produced in such tubes could result from the fermentation of the sugars from the juice by microorganisms which are unable to attack lactose. It is a common experience for the citrus products microbiologist to find high presumptive coliform indices in orange samples which are either negative for the completed coliform test or have a low coliform index. Martinez and Appleman (1949) noted that a large number of positive presumptive tests resulted when coliform determinations were run on 62 samples of frozen orange juice from which no coliforms were isolated. Although they did not discount the possibility that fermentation of the orange juice sugars might have caused some of their spurious results, they attributed many of their positive presumptives to lactose-fermenting yeasts which produced a sheen on eosin-methylene blue agar.

Wolford (1950) found that the presumptive coliform index in 78 out of 79 samples tested was more than ten times the completed index obtained. In a later study, Wolford (1954) obtained 2,232 positive presumptives out of a total of 3,422 lactose broth tubes inoculated with orange juice or surface rinses of oranges. False positives accounted for 1,297 of the 2,232 positives. Fermentation of the orange juice sugars was postulated as playing a part in the production of the many false positive presumptive tests.

The present paper reports on an investigation undertaken to determine the extent to which fermentation of the sugars and/or other substances in the juice may play a part in the presumptive test for coliform organisms in orange juice.

Experimental Methods

Commercial, unheated frozen orange concentrate was reconstituted to single strength with sterile water containing sufficient NaOH to raise the pH of the juice to 6.0. Standard lactose broth and nutrient broth fermentation tubes were inoculated in parallel with 1-ml and 0.1-ml amounts of the reconstituted juice, and incubated at 37 C for 48 ± 3 hours. Nutrient broth was compared with lactose broth, the standard presumptive coliform medium, because it differs from the latter only in that it contains no fermentable sugar and, therefore, any gas produced in the tubes of the former must come from fermentation of the constituents of orange juice. Material from gassing tubes was streaked on Levine’s eosin methylene blue agar plates which were incubated overnight at 37 C. Typical coliform or coliform-like colonies were transferred to lactose broth fermentation tubes and to nutrient agar slants. Gas production after 48 ± 3 hours at 37 C by gram negative, nonsporulating rods confirmed the presence of coliform bacteria. This conforms to the procedure prescribed for the completed coliform test in Standard Methods for the Examination of Water and Sewage (A.P.H.A., 1946).

Results and Discussion

Gas was formed in both the lactose and nutrient broth tubes. The results obtained are shown in table 1. While gas was produced in more lactose broth tubes (57.6 per
cent) the difference is not great, being little, if any, greater than differences inherent in the experimental method. In both media, positive presumptive tests greatly outnumbered the completed tests when the analyses were carried through the latter step. The somewhat higher recovery of coliforms from lactose broth indicates that lactose may exert an enriching action which favors growth of the coliforms. However, the fact that gas was produced in both media indicates that, when coliform determinations are run on orange juice, positive presumptive tests do not necessarily indicate that coliforms are present or even that lactose has been fermented.

In water analysis, it is permissible to confirm only the positive presumptives in the two highest dilutions and to assume that coliforms are present in all gassing tubes of the lowest dilution of the recommended dilution series described in Standard Methods for the Examination of Water and Sewage 9th ed., paragraph C of the section on Coliform Determinations (A.P.H.A., 1946). However, when orange juice is tested for its coliform index, all positives should be confirmed. Spurious results may be obtained when nonlactose fermenters, such as yeasts and heterofermentative lactobacilli, attack the sugars of orange juice. Martínez and Appleman (1949) observed that occasionally lactose-fermenting yeasts will give false positive presumptive tests. Microscopic examination, prescribed in the completed coliform test, precludes the confusing of such organisms with coliforms, and the completed coliform test should always be made when the coliform index of orange juice is determined.


cents

table 1. comparison of lactose and nutrient broths for presumptive coliform determination in orange juice

<table>
<thead>
<tr>
<th>Volume of Inoculum</th>
<th>Total Tubes</th>
<th>Lactose Broth</th>
<th>Nutrient Broth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive presumptives</td>
<td>Confirmed coliforms</td>
</tr>
<tr>
<td>ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>125</td>
<td>106</td>
<td>30</td>
</tr>
<tr>
<td>0.1</td>
<td>125</td>
<td>38</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td>144</td>
<td>48</td>
</tr>
</tbody>
</table>

Hunter (1939) wrote that “determination of the presence of coliform organisms does serve a very useful purpose when there has been sufficient study of the methods of production and handling of the class of food product under control to lay the basis for interpretation of the significance of members of the coliform group as a whole or of genera or species within the group. Until this basis is laid, the very ubiquity of the group places a handicap upon the interpretation of results obtained by laboratory examination.” To date, the literature on sources and significance of coliforms in frozen citrus products is too limited to ascribe any indisputable significance to coliforms in the products. Sanitation methods practiced in the production of frozen, concentrated orange juice are, for the most part, thorough and comprehensive (Beisel, 1951; Brokaw, 1952). Even so, many anomalous results are found by every worker who has investigated coliform bacteria in frozen citrus products and one wonders about the value of applying the coliform test to the product. However, if it must be applied, the preceding results indicate that attaching any great significance to the presumptive coliform test in orange juice shows a disregard for the chemical nature of the product.

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