RELATIVE EFFICIENCIES OF SEVERAL LIQUID PRESUMPTIVE MEDIA USED IN THE MICROBIOLOGICAL EXAMINATION OF CITRUS JUICES[3]

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Introduction

According to the definition given in the latest edition of *Standard Methods of Water Analysis* (2), the coliform group of organisms should be considered as including "all aerobic and facultative anaerobic, Gram-negative, nonsporeforming bacilli which ferment lactose with gas formation." Although the presence and estimation of the number of these organisms in water has become an integral part of the bacteriological procedure for judging the quality of water, the significance of their presence in citrus and other food products is not so clearly understood. Since Wolford (3) found organisms resembling *Escherichia coli* in citrus juices, it is probable that further work will be done in an effort to determine the incidence and significance of these organisms in citrus juices. It is essential, therefore, that a standard enrichment medium for the detection of these organisms in citrus juices be adopted for use in future investigations. Fortunately, the citrus industry has the advantage of knowledge gained through the almost countless numbers of investigations which were directed toward the selection of a standard presumptive medium for the examination of water.

During the years following 1923, at which time the Committee on Standard Methods of the American Public Health Association adopted a 0.5 percent lactose broth as the standard presumptive medium, many enrichment media have been proposed to replace it. Since the ideal medium would be one which would prove favorable to growth of the coliform group and unfavorable to all other organisms, these media have been thoroughly evaluated from this viewpoint. Before any medium will be recommended to replace standard lactose broth, it must be shown that the medium in question will give fewer gas-positive presumptive tests than lactose broth and, at the same time, yield a higher percentage of coliform isolations. Thus far, only one medium, lauryl tryptose broth, has satisfied these requirements and this only as a preliminary enrichment medium for certain types of water.

For obvious reasons, the adoption of a routine medium for the detection of coliforms in citrus products should require the same careful thought. Logically the nature of the product would suggest that methods suitable for the analysis of water would not necessarily be the most satisfactory in this case.

In order to obtain a sufficient sample for analysis, a relatively large amount of sugars other than lactose must be added to the medium. For example, according to the procedure recommended by Beisel and Troy (1) for using the Vaughn-Levine boric acid medium, 25 ml. of a 42° Brix concentrate was reconstituted to 100 ml. with 75 ml. of a concentrated medium. In this case, the resulting medium contained, in addition to the customary 0.5 percent lactose, at least 5 percent of sucrose and invert sugars. Similarly, if only 1 ml. of a

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reconstituted concentrate is added to 9 ml. of medium, there is still present in the final medium between 0.5 and 1.0 percent of these sugars. Although the first presumptive medium that was adopted by the Committee on Standard Methods contained 1 percent glucose, it was later shown that many bacteria of little or no sanitary significance were able to ferment this sugar and the more selective lactose was substituted. Also the original concentration of 1 percent was lowered to 0.5 percent when it was shown that *E. coli* might be killed by the acidity produced from the higher concentration.

For the purpose of selecting an enrichment medium for the detection of coliform organisms in citrus juices, the picture is further complicated by the fact that the juices usually contain relatively large numbers of yeasts, many of which are capable of fermenting sucrose or hexoses with the formation of visible gas.

The purpose of this work was to evaluate several possible enrichment media on the basis of their ability to (1) Suppress the growth of yeasts, particularly those gas-producing species, and (2) Allow the detection of coliform organisms even though they are present in relatively small numbers.

Experimental

The necessity for this study was pointed out by the results of a preliminary survey which was carried out during the 1949-50 season. During this survey 48 samples of 42° Brix orange concentrate, including 12 samples which were obtained from Wolford, were tested using standard lactose broth, brilliant green lactose bile and the Vaughn-Levine boric acid medium. For purposes of comparison and inoculum to medium ratio suggested by Beisel and Troy was used for all media. Similarly, all tests were run in duplicate, one set being incubated at the customary 37° C. which is recognized by Standard Methods while the other was incubated at 43° C., the temperature recommended for the boric acid medium. All presumptive tests showing any amount of gas within 48 hours were confirmed by the usual methods. Results of this survey are presented in Table 1.

On the basis of this short survey it appears that standard lactose broth, although yielding a greater percentage of false positive reactions than either of the other media, is a more efficient enrichment medium for the isolation of these organisms. It is also apparent that increasing the incubation temperature from 37° C. to 43° C. decreases the number of positive presumptive tests. More significant, however, is the fact that the percentage of positive confirmations was also decreased at the higher temperature. The complete lack of confirmation in the case of the boric acid medium should not

TABLE 1.
COMPARISON OF LACTOSE BROTH, VAUGHN-LEVINE BORIC ACID BROTH AND BRILLIANT GREEN LACTOSE BILE AS ENRICHMENT MEDIA FOR COLIFORM ORGANISMS IN 42° BRIX ORANGE CONCENTRATE.

	Standard Lactose Broth		Vaughn-Levine Boric Acid Medium		Brilliant Green Lactose Bile	
	37°C.	43°C.	37°C.	43°C.	37°C.	43°C.
Total number of samples	48	48	48	48	48	48
Number of positive presumptive tests	48	33	34	14	37	9
Number of positive tests confirmed	8	4	0	0	3	2
Percentage of total tests confirming	16.7	8.3	0	0	6.2	4.2

be construed to mean that this medium inhibits these particular organisms because such is not the case. All organisms which were isolated from either of the other two media and which resembled *E. coli* to any degree on eosin methylene blue agar were capable of producing gas in the boric acid medium at 43° C. when grown in pure culture.

The fact that these organisms did not show up in the boric acid medium under the conditions of this experiment does suggest, however, that more work should be done before this or any medium is selected as a standard enrichment medium for the examination of citrus juices.

The data in Tables 2 and 3 illustrate the relative merits of several possible media for separating yeasts and coliforms. Fifty strains of yeasts isolated from orange juice and 16 strains of *E. coli* obtained from various state health laboratories were used in this study. Standard lactose broth, brilliant green bile 2 percent, lauryl tryptose broth and the boric acid medium were compared. Glucose in an amount of 0.5 percent was added to each of the media in order to provide a fermentable sugar other than lactose. This was done in an effort to more nearly simulate conditions obtained when concentrate is added to a presumptive medium.

As shown in Table 2, 12 of a total of 50 yeast strains were capable of fermenting sugars in the lactose broth to gas when incubated at 37° C. for a period of 48 hours. Brilliant green lactose bile, while inhibiting some of the non-gas producing yeasts, did not inhibit the gas-formers over a period of 48 hours. In the case of lauryl tryptose broth, however, only 3 of the 12 strains produced gas

TABLE 2.

GROWTH AND GAS PRODUCTION BY STRAINS OF YEASTS AND *ESCHERICHIA COLI* IN SEVERAL LIQUID PRESUMPTIVE MEDIA AT 37° C.

YEASTS

	Standard Lactose Broth		Brilliant Green Lactose Bile		Lauryl Tryptose Broth		Vaughn-Levine Boric Acid Broth	
Hours Incubation	24	48	24	48	24	48	24	48
Total strains	50	50	50	50	50	50	50	50
Number growing	36	42	23	25	22	23	6	6
Percent growing	72.0	84.0	46.0	50.0	44.0	46.0	12.0	12.0
Number gasing	10	12	3	12	0	3	0	0
Percent gasing	20.0	24.0	6.0	24.0	0	6.0	0	0

E. COLI

	Standard Lactose Broth		Brilliant Green Lactose Bile		Lauryl Tryptose Broth		Vaughn-Levine Boric Acid Broth	
Hours Incubation	24	48	24	48	24	48	24	48
Total strains	16	16	16	16	16	16	16	16
Number growing	16	16	16	16	16	16	11	16
Percent growing	100.0	100.0	100.0	100.0	100.0	100.0	68.8	100.0
Number gasing	16	16	16	16	16	16	11	14
Percent gasing	100.0	100.0	100.0	100.0	100.0	100.0	68.8	87.5

TABLE 3.
GROWTH AND GAS PRODUCTION BY STRAINS OF YEASTS AND *ESCHERICHIA COLI* IN SEVERAL LIQUID PRESUMPTIVE MEDIA AT 43° C.

YEASTS

	Standard Lactose Broth		Brilliant Green Lactose Bile		Lauryl Tryptose Broth		Vaughn-Levine Boric Acid Broth	
Hours Incubation	24	48	24	48	24	48	24	48
Total strains	50	50	50	50	50	50	50	50
Number growing	13	13	3	3	8	10	1	1
Percent growing	26.0	26.0	6.0	6.0	16.0	20.0	2.0	2.0
Number gasing	0	9	0	0	0	0	0	0
Percent gasing	0	18.0	0	0	0	0	0	0

E. COLI

	Standard Lactose Broth		Brilliant Green Lactose Bile		Lauryl Tryptose Broth		Vaughn-Levine Boric Acid Broth	
Hours Incubation	24	48	24	48	24	48	24	48
Total strains	16	16	16	16	16	16	16	16
Number growing	16	16	16	16	16	16	16	16
Percent growing	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Number gasing	10	12	5	7	14	14	14	14
Percent gasing	62.5	75.0	31.25	43.75	87.5	87.5	87.5	87.5

in 48 hours while the boric acid medium completely inhibited all but 6 of the 50 strains and none of these were gas-producers. On the other hand, all 16 strains of *E. coli* grew and produced gas within 24 hours at 37°C. in all media except the boric acid medium. In this medium, 11 of the 16 strains grew within 24 hours and 14 produced gas within 48 hours.

As shown in Table 3, completely different results were obtained at an incubation temperature of 43°C. with only lactose supporting gas production by yeasts at this temperature. However, the advantage of increased inhibition of yeasts at the higher temperature is minimized by a corresponding inhibition of *E. coli* in all of the media except the Vaughn-Levine medium.

Discussion

On the basis of these results, none of the four media which were compared are completely satisfactory for the purpose of eliminating false positive presumptive tests and for the detection of the presence of coliforms. It is also probable that in the majority of instances citrus juices will contain as many or more yeasts and fewer coliforms than were used for inocula in these experiments.

The most important criterion for evaluating a presumptive medium is its ability to support the growth of coliform organisms as well as inhibit other organisms. Therefore any medium recommended as a standard enrichment medium for citrus juices should first be carefully compared with a medium known to be capable of detecting the presence of coliforms. Similarly, it is important that such a comparison be made under natural conditions, since the use of pure cultures ignores such factors as the possible presence in citrus juices of substances which are slightly toxic to coliform organisms.

The presence of such substances might be particularly significant in the case of media containing inhibitors in concentrations slightly below a level at which coliforms themselves are affected. These factors, in combination with an incubation temperature above the optimum for coliforms, could explain the inability of selective media to detect these organisms in some instances.

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STORAGE CHANGES IN CITRUS MOLASSES

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Introduction

Citrus molasses, which has become a familiar livestock feed in Florida has been produced commercially for less than ten years. Its wide acceptance and increasing popularity warrant more complete understanding of its physical and chemical properties. Buyers of this carbohydrate concentrate are interested in obtaining further information regarding the product, storage changes, and the ramifications of microorganisms associated with it.

Citrus molasses is produced from the rinds and pulp of citrus after the juice has been expressed. This waste residue is chopped, limed, and pressed to yield a press liquor of 10°-14° Brix (percent soluble solids content by weight) which when concentrated to 72°-75° Brix is the final molasses. Since citrus molasses can be produced only during the processing season, the processor is required to store millions of gallons to serve the year round needs of cattlemen. Certain changes take place during storage and they are the subject of this report to industry.

Before discussing storage changes in citrus molasses it might be well to examine Table 1 wherein the comparative analysis between this product and the common molasses obtained from sugar refining is presented. The average analysis for clarified citrus molasses represents samples made from several varieties of both grapefruit and orange. Clarified molasses refers to a product made from a clear press liquor. It is immediately noticeable that blackstrap is generally concentrated to a higher degree Brix, but has the disadvantage of having more than a proportionately higher ash content. Citrus molasses producers tend now to use 72° Brix as a minimum value with the average being maintained at a higher level.

Sugar Losses and Instability During Storage

In storage, citrus molasses has been found to slowly undergo both a physical and chemical transformation. Of paramount importance are the changes in sugar content which occur on storage. When ten samples of citrus molasses collected from ten commercial processors in January of 1948 were reanalyzed by the Lane-Eynon Volumetric procedure they were found to have lost

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