Method Application: MicroSnap® Total with Low pH Tea Beverages

Introduction

Low pH/high acid products are challenging product matrices. Such samples can interfere with detection and enumeration of microbes and often require sample neutralization prior to analysis. By diluting the low pH beverages with BPW and increasing the time to detection to 8 hours, we were able to neutralize the sample effects and see detection of total viable bacteria using MicroSnap® Total and MicroSnap Enhanced nutrient broth products within 8 hours, shortening time to results and simplifying the process.

MicroSnap Total is a rapid bioluminogenic test for the detection and enumeration of total aerobic bacteria in foods and beverages. The MicroSnap Total test is comprised of an enrichment step that promotes the growth of aerobic bacteria and a detection device that contains a bioluminogenic reagent used to obtain results in relative light units. The relative light units (RLU) values are then directly correlated to colony forming units (CFU) using the conversion chart found in the product insert.

Objective

To determine the optimal steps for sample preparation of low pH tea beverages for microbial analysis using MicroSnap Total and MicroSnap Enhanced Nutrient Broth.

Equipment, Supplies, and Reagents

- MicroSnap Total Enrichment Devices
- MicroSnap Enhanced Nutrient Broth
- MicroSnap Total Detection Devices
- Incubator – set at 30 ˚C ± 2 ˚C
- EnSURE® Touch luminometer
- Buffered Peptone Water (BPW)
- Tryptone Soy Broth (TSB)
- Tryptone Soy Agar (TSA)
- *Staphylococcus aureus* ATCC 6538
- *Bacillus cereus* ATCC 11778
- *Escherichia coli* ATCC 8739
- Pipettes and tips
- Cell spreaders
- Vortex mixer

Ovenight Culture Enrichment and Preparation

Cultures were prepared by aliquoting 5 mL of TSB into a sterile vial and inoculating it with a colony of the bacteria. Each bacterial culture was then incubated overnight at 37 ˚C. On the day of testing, a mixed culture was prepared by pipetting 1 mL of each overnight culture into a new sterile vial and homogenizing using a vortex.

Beverage Preparation

Matrix Effects – A 10% dilution of each beverage was prepared in BPW by transferring 5 mL of the beverage to a sterile container with 45 mL of BPW. This dilution neutralized the pH of the product and diluted nonmicrobial ATP naturally present in the beverages.

Spiking Study – For each beverage, 10% dilutions were prepared in BPW by transferring 5 mL of the beverage into a sterile container with 45 mL of BPW; these were used for testing with MicroSnap Total. Additionally, 20% dilutions of each beverage were prepared in BPW by transferring 10 mL of the beverage into a separate sterile container with 40 mL of the BPW; these were used for testing with MicroSnap Enhanced Nutrient Broth.

Method

Matrix Effects

For each sample type, 1 mL of the undiluted beverages and 1 mL of the 10% dilutions were pipetted into separate MicroSnap Total Enrichment devices; the devices were activated and incubated at 30 ˚C for 8 hours ± 10 minutes. After the incubation period, the enriched samples were tested using MicroSnap Total Detection devices. In parallel, the beverage dilutions were plated onto TSA to confirm the absence of microbial contamination.

Spike Study in MicroSnap Enrichment Swabs

For each beverage, 10% dilutions were prepared in BPW; these dilutions were utilized to prepare serial dilutions of the mixed microbial culture. For each culture, 1 mL of each dilution was pipetted into MicroSnap Total Enrichment swabs; the devices were activated and incubated at 30 ˚C for 8 hours. The culture dilutions were also plated onto TSA and incubated for 24 hours at 30 ˚C. At the end of the 8-hour incubation period, the enriched samples were tested with the MicroSnap Total Detection devices. The following day, colony counts were performed on the TSA plates. Log values were then calculated for the colony counts and RLU values obtained from MicroSnap Total. These were compared for correlation.

Spike Study in MicroSnap Enhanced Nutrient Broth

For each beverage tested, 20% dilutions were prepared in BPW; these dilutions were used to prepare serial dilutions of the mixed microbial culture. For each culture, 1mL of each dilution was pipetted into MicroSnap Enhanced Nutrient Broth; the vials were then vortexed and incubated at 30 ˚C for 8 hours. The mixed culture serial dilutions were also plated onto TSA and incubated for 24 hours at 30 ˚C. At the end of the 8-hour incubation period, the enriched samples were tested using MicroSnap Total Detection devices. The following day, colony counts were performed on the TSA plates and compared to the corresponding RLU values from the MicroSnap device.

Results and Discussion

Matrix Effects

All beverages were found to be acidic with pH values ranging from pH (3.17- 4.23) as shown in Table 1. Diluting the samples in BPW helped neutralize the low pH. All beverages were also found to have a high background signal caused by high nonmicrobial ATP naturally present in the beverages; this signal was also eliminated by the dilution prepared in BPW as shown in Table 2. Plate counts for all beverage dilutions were zero, confirming that the background signal was due to nonmicrobial ATP.

Spike Study in 10% Dilutions using MicroSnap Total

The R^2 values between the log RLU and log CFU were found to be >0.96 for all beverages, demonstrating a good correlation between the MicroSnap Total and Plate methods (Figure 1).

Spike Study in 20% Dilutions using MicroSnap Enhanced Nutrient Broth

The R^2 values between the log RLU and log CFU values were found to be ≥ 0.97 for all beverages, demonstrating a good correlation between the Enhanced Nutrient Broth and Plate methods (Figure 2).

Table 1. pH Results of Neat and Diluted Products

Beverage	Neat	Neat in Nutrient Broth	10% in BPW
Grapefruit	3.43	4.93	6.78
Sweet Tea	4.23	6.70	7.09
Blueberry	3.17	6.52	7.07
Peach	3.28	6.55	7.08
Strawberry	3.27	6.49	7.07

Table 2. Background Signal after 8-hour Incubation in MicroSnap Total Enrichment Swab Media

Beverage	Undiluted (CFU/mL estimate*)	10% in BPW (CFU/mL estimate)
Grapefruit	1,126	<10
Sweet Tea	1,930	<10
Blueberry	64	<10
Peach	9,037	<10
Strawberry	3,248	<10

*Estimate can have up to a ± 30% error

Figure 1. Correlation Between MicroSnap and Plate Counts (RLUs vs CFUs)

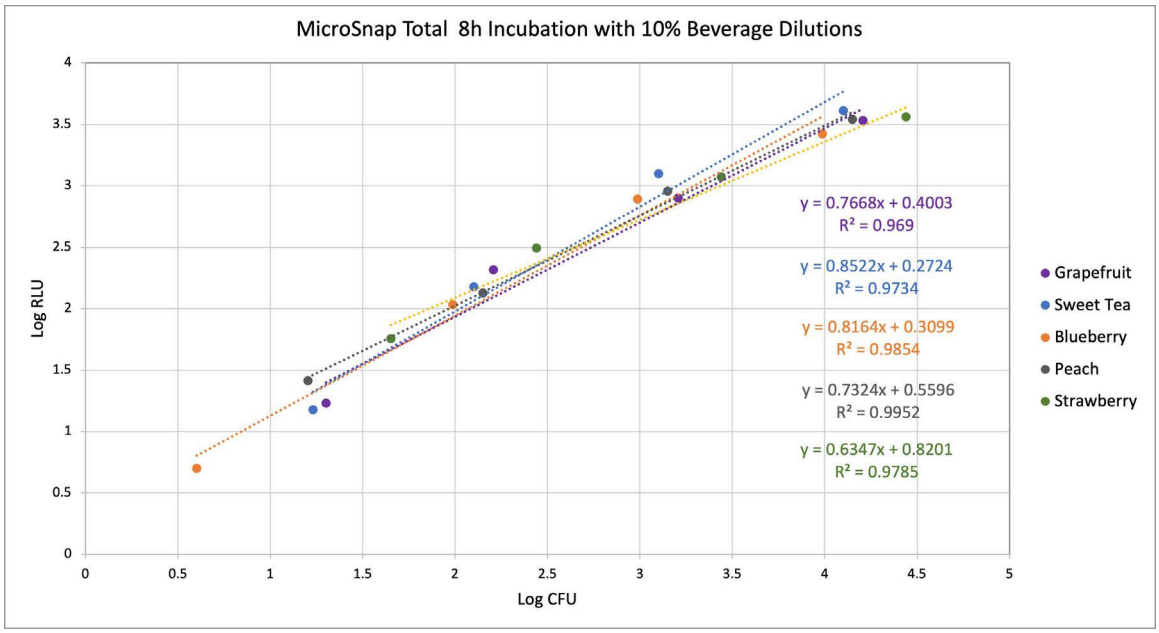
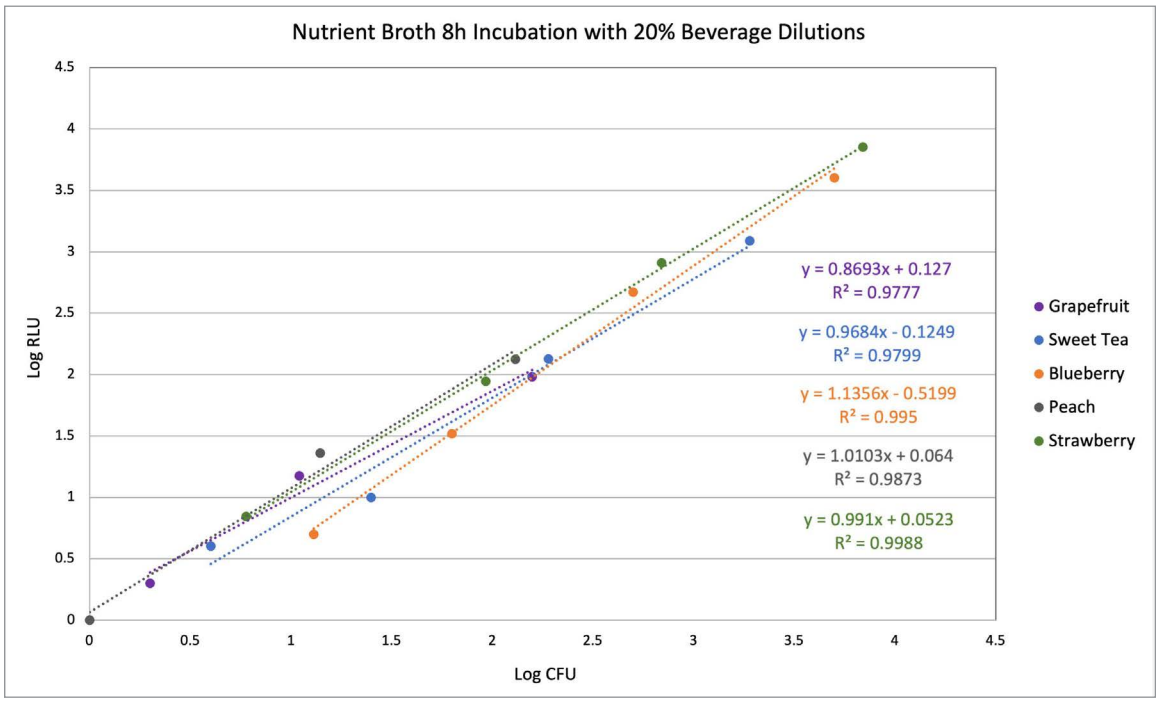


Figure 2. Correlation Between Growth in Enhanced Nutrient Broth and Plate Counts



Conclusions

The MicroSnap technology demonstrated that total viable bacterial detection is feasible from low pH tea beverages within 8 hrs. The data shows equivalent performance between MicroSnap and the traditional agar plating method, which has a time-to-result of at least 24-48 hours. It was determined that dilution of tea beverages in BPW, neutralized the sample effects and enabled the detection of bacteria within 8 hours.

The results show that MicroSnap Total and MicroSnap Enhanced Nutrient Broth technologies can be used as a simple, accurate and faster method for the measurement of total bacterial count within an 8-hour time frame.