

Comparison of Petrifilm™ and Standard Pour Plate method for the Analysis of Fruit Juices in Guyana

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Abstract: The aim of this study was to analyze the quality of fruit juices using conventional pour plate method and Petrifilm method. The Petrifilm aerobic plate count developed by 3MTM Laboratories is a ready to use system for the enumeration of aerobic bacteria in different foods. A total of 31 unpasteurized juices, 31 pasteurized juices and 31 water samples were analyzed to correlate the methods independently. The total viable bacteria count ranged from >300 to 1 CFU/ml by both pour plate method and Petrifilm method in the unpasteurized juices samples. The comparison of water sample with pour plate method against the Petrifilm method showed a significant correlation (0.998, p-value < 0.05). Mean microbial count in pour plate method was 126.5CFU/ml and mean microbial count in petrifilm method was 129.9CFU/ml. Brix, pH and total acidity of the juice didn't show any effect on the method of analysis. Regardless of the type of juice, the Petrifilm was found to be comparable with the conventional method in analyzing total aerobic bacteria from fruit juices and method can be used widely in Guyana or other countries.

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1. Introduction

Fresh or unpasteurized juices are widely consumed in developing countries. In Guyana, fresh juices are widely consumed because of their nutritional benefits and low cost. Most common and widely used juices are Cherry, Passion fruit, Fruit punch and Orange. Fresh Juices are fruit juices squeezed or pulped and diluted with or without sugar. These are widely available at most food outlets around the capital city, Georgetown.

Pathogenic organisms can enter fruits and vegetables through damaged surfaces, such as punctures, wounds, cuts and splits that occur during growing or harvesting. Contamination from raw materials and equipment, additional processing conditions, improper handling, prevalence of unhygienic conditions contribute substantially to the entry of bacterial pathogens in juices prepared from these fruits or vegetables (Victorian Government Department of Human Services 2005; Oliveira *et al.*, 2006; Nicolas *et al.*, 2007). Most local fruit juice vendors are not educated in the areas of food safety and do not know the public health implications of serving contaminated fruit juices.

In the fruit juice industry, emphasis is placed on the microbiological testing for rapid results to meet the demands of the market. Rapid microbiological testing is becoming more popular in the food industry. Food industries depend on the accuracy of rapid testing on the release of their products since false negative can cost a company to waste resources on product recall and lawsuit from consumers, while false positives can result in time and resources wasted in expensive retesting (3M MicroMessenger, Volume

111 199). Enumeration of these microorganisms is an important aspect of evaluating the microbiological quality of acidic foods. However, the most frequently used analytical method for enumeration is pour-plating in non-selective and selective culture media (Maturin *et al.*, 2001; Tourna *et al.*, 2001), which is both time-consuming and labor-intensive (Feng, 2001). In Guyana, most established juice industries use the conventional pour plate method for the analysis of juices. The purpose of this research was to validate the 3M Petrifilm against the conventional pour plate method for the enumeration of aerobic bacteria and aciduric bacteria in juices.

2. Materials and Methods

2.1 Collection of samples:

Juice samples were collected randomly from different juice vendors and distribution trucks. The distribution trucks were sampled directly from the manufacturers, whilst the vendors were retailers of the juices. Samples were also randomly collected from the Georgetown area. A total of thirty one samples were collected and analyzed using both the conventional pour plate and the Petrifilm methods. Five variety of juices were collected namely Cherry, Fruit Punch, Passion fruit, Cherry Passion, and Guava based on the fruit in season. Samples were collected in sterile whirlpak bags and analyzed within one hour of collection. Water samples were also collected randomly from different water sources. Samples were collected in sterile whirlpak bags. A total of thirty one samples were obtained and analyzed using both methods.

2.2 Sample Analysis:

The sample was mixed and 1 ml was placed on the Petrifilm and spread as per the manufacturer's instructions and 1ml was placed in a sterile petri dish and 10- 15ml Orange Serum Agar (OAS) was added mixed and incubated. OSA plates were incubated at 30°C for 48 hours. After 48 hrs incubation the plates were removed and all the colonies were counted. The Petrifilm was incubated at 35°C for 48 hours. After the incubation period the colonies were counted and reported as the number of colonies present. Pasteurized juice samples were used as the constant sample.

Since the juices were of different colors this would influence the visual colonies so water samples were used as another constant to compare the performance of two methods as well. Colonies were counted using the Quebec Colony Counter and the Stereo Microscope. Colonies were reported as CFU/ml of sample. The physical and chemical variables were determined by measurement of pH with a pH-meter, total titratable acidity by titration with 0.1 N sodium hydroxide solution (results expressed in grams citric acid per 100mL of sample) and quantity of total soluble solids (°Brix) with a 0-32 °Brix refractometer.

3. Results

A total of thirty one samples unpasteurized and pasteurized were collected and analyzed using both the conventional pour plate and the petrifilm methods. Five variety of juices were collected namely Cherry, Fruit Punch, Passion fruit, Cherry Passion, and Guava based on the fruit season.

Table 1 shows the analysis of unpasteurized fruit juices using conventional method and Petrifilm method. Total colony count by Petrifilm compared to conventional method had a minimum of 1CFU/ml to 300CFU/ml. The pH of all the fruit juices was found to be between 2.91 and 3.76 while the total acidity were between 1.6 to 4.6 ranges. Correlation of pour plate method to Petri plate method in unpasteurized juice has showed a significant correlation = 0.95 and p value < 0.05. The results also showed a significant difference between pour plate method and Petrifilm method in pasteurized juices with correlation 0.97 and p value < 0.05. Microbial count was seen only in cherry juice with 1-3CFU/ml. Analysis of water sample using conventional and Petrifilm method is shown in Table 3.

Petrifilm method showed significantly higher microbial growth (mean=129.9 CFU/ml) compare to pour plate method (mean=126.5 CFU/ml) with a correlation 0.998 and p value <0.05. Table 4 shows the analytical results of both pasteurized and unpasteurized juices with pour plate method and Petrifilm method.

Table 1: Comparison of Pour plate and Petri film method in isolation of microbes from Unpasteurized fruit juices along with Physical and chemical characteristics in Guyana.

Samples	Pour Plate	Petri Film	Acidity	pH	Brix (°Bx)
FP 1	120	150	1.56	3.61	10.0
FP 2	108	120	1.56	3.61	10.0
FP 3	100	110	1.56	3.61	10.0
FP 4	60	70	2.45	3.36	9.3
FP 5	180	200	2.76	3.71	9.8
FP 6	220	240	2.76	3.71	9.8
FP 7	280	300	2.76	3.71	9.8
FP 8	11	10	2.76	3.71	9.8
FP 9	4	4	4.6	3.47	12.1
FP10	1	1	4.01	3.44	11.0
PF 1	12	10	3.9	3.03	9.9
PF 2	8	6	3.9	3.03	9.9
PF 3	4	4	3.9	3.03	9.9
PF 4	140	138	3.84	3.32	10.9
PF 5	8	9	4.6	3.21	11.4
PF 6	3	3	4.6	2.91	12.0
CP 1	90	85	3.62	3.29	11.1
CP 2	120	80	3.62	3.30	11.8
CP 3	180	200	3.62	3.30	11.8
CP 4	120	140	3.68	3.32	11.4
CP 5	150	165	3.76	3.32	11.8
GP	10	11	2.31	3.76	8.80
Ch 1	300	300	3.84	3.36	11.2
Ch 2	300	300	3.84	3.36	11.2
Ch 3	300	300	3.84	3.36	11.2
Ch 4	80	89	4.26	3.51	11.4
Ch 5	300	150	4.33	3.30	10.5
Ch 6	300	200	4.33	3.30	10.5
Ch 7	300	230	4.33	3.30	10.5
Ch 8	10	10	4.33	3.30	10.5
Ch 9	1	1	4.33	3.35	12.3

Table 2 shows the comparison of pour plate method against petrifilm method in analysis of pasteurized juices.

Samples	Pour CFU/ml	Plate Petri CFU/ml	Plate
FP 1	0	0	
FP 2	0	0	
FP 3	0	0	
FP 4	0	0	
FP 5	0	0	
FP 6	0	0	
FP 7	0	0	
FP 8	0	0	
FP 9	0	0	
FP 10	0	0	
Ch 1	2	3	
Ch 2	2	2	
Ch 3	1	2	

Samples	Pour Plate CFU/ml	Petri CFU/ml
Ch 4	0	0
Ch 5	0	0
Ch 6	0	0
Ch 7	0	0
Ch 8	0	0
Ch 9	0	0
PF 1	0	0
PF 2	0	0
PF 3	0	0
PF 4	0	0
PF 5	0	0
PF 6	0	0
CP 1	0	0
CP 2	0	0
CP 3	0	0
CP 4	0	0
CP 5	0	0
GP	0	0

Table 3: Comparison of Pour plate method and Petri film in isolation of microbes in different water samples.

Water Sample	Pour Plate CFU/ml	Petri Film CFU/ml
Sam 1	200	208
Sam 2	120	125
Sam 3	80	87
Sam 4	98	105
Sam 5	65	70
Sam 6	100	100
Sam 7	112	112
Sam 8	85	86
Sam 9	48	54
Sam 10	160	162
Sam 11	180	182
Sam 12	140	146
Sam 13	90	93
Sam 14	120	123
Sam 15	180	185
Sam 16	19	24
Sam 17	160	169
Sam 18	143	147
Sam 19	186	189
Sam 20	145	147
Sam 21	180	188
Sam 22	224	225
Sam 23	213	213
Sam 24	111	111
Sam 25	102	102

Sam 26	84	86
Sam 27	109	108
Sam 28	142	146
Sam 29	38	42
Sam 30	172	17
Sam 31	116	118

Table 4: Analytical report of pasteurized and unpasteurized juice by pour plate and Petrifilm method.

Mean Unpasteurized Sample / Pour Plate Method CFU/ml						
Unpasteurized Sample	Mean	N	SD	Min.	Max.	Range
Cherry sample	210.1	9	136.6	1	300	299
Fruit Punch	108.4	10	95.2	1	280	279
Cherry Passion	132.0	5	34.2	90	180	90
Passion Fruit	29.2	6	54.4	3	140	137
Guava	10.0	1	-	10	10	0

Mean Unpasteurized Sample / Petrifilm Method CFU/ml						
Unpasteurized Sample	Mean	N	SD	Min.	Max.	Range
Cherry sample	175.6	9	120.4	1	300	299
Fruit Punch	120.5	10	103.5	1	300	299
Cherry Passion	134.0	5	51.6	80	200	120
Passion Fruit	28.3	6	53.8	3	138	135
Guava	11.0	1	-	11	11	0

Mean Pasteurized Sample / Pour plate Method CFU/ml						
Pasteurized Samples	Mean	N	SD	Min.	Max.	Range
Cherry sample	0.78	9	1.20	0	3	3
Fruit Punch	0	11	0	0	0	0
Cherry Passion	0	5	0	0	0	0
Passion Fruit	0	5	0	0	0	0
Guava	0	1	0	0	0	0

Mean Pasteurized Sample / Petrifilm plate Method CFU/ml						
Pasteurized Samples	Mean	N	SD	Min.	Max.	Range
Cherry sample	0.56	9	0.88	0	2	2
Fruit Punch	0	11	0	0	0	0
Cherry Passion	0	5	0	0	0	0
Passion Fruit	0	5	0	0	0	0
Guava	0	1	0	0	0	0

4. Discussion

Street vended fruit juices are well appreciated by the consumers because of their taste, low price, and availability at right time (Ohiokpehai, 2003). In spite of the potential benefits offered by fruit juices, concerns over their safety and quality have been raised; as freshly prepared juices have no process or steps to minimize the microorganisms if they are contaminated (Mahale *et al.*, 2008).

In the present study the juice samples were collected from most populated areas of Georgetown. These samples show alarmingly high bacteria counts in fresh or unpasteurized juices. Based on the analysis carried out, the conventional pour plate was compared against the Petrifilm showed high mean bacterial count with Petrifilm method.

Brix, pH and total acidity had no effect on the media used for culturing whether it was Orange Serum Agar pour plate or Petrifilm. However, it affected the amount of aciduric bacteria present in the juice. A more acidic juice will have a lower number of bacteria compared to a juice with lower acidity. Juices that have a higher Brix were shown to have more bacteria. The Brix was directly proportional to the number of bacteria present. This can be due to the fact that higher Brix indicate higher sugar content (Pilo *et al.*, 2009).

Unpasteurized juices showed a higher number of bacteria as compared with pasteurized juices using both methods. This is because pasteurized juices are treated and the bacterial load is reduced by 10 log. Unpasteurized juices have higher bacteria counts due to contamination occurring during production, handling and storage. Improper washing of fruits add bacteria to juices leading to contamination. In addition lack of appreciation of basic safety issues by vendors contribute to augmentation of the microbial loads (Mahale *et al.*, 2008). The juices which showed a higher bacteria counts were cherries or juices that contain cherries as part of their formula. This may be due to the delicate nature of the fruit. The skin is easily damaged and this enables microorganisms to get in the fruit and contaminate it easily. Passion fruit on the other hand showed the least amount of contamination due to the hardness of the skin, which protects it from environmental contaminants. Other sources of contamination in fruit juice can include unavailability of running water for dilution and washing, prolonged preservation without refrigeration, unhygienic surroundings with swarming flies and airborne dust (Lewis *et al.* 2006).

In comparison, pasteurized fruit juices sold in the market showed little or no microbial contamination and appeared clean and safe for human consumption. Due to such heavy microbial

contamination, attempts can be made to decontaminate or to reduce the microbial load of the fruit juice. Various thermal and non thermal treatments were found to be effective in decontaminating the fruit juices (Raso *et al.* 1998; Yen and Lin 2003).

Other studies on comparative evaluations between petrifilm and conventional methods to determine the microbial quality of different foods showed remarkable correlation between the two methods, $r = 0.989$ (Chain and Fung, 1991; Beuchat *et al.*, 1998), $r = 0.97$ (Mizuochi and Kodaka, 2000). Blackburn *et al.*, 1996 found a correlation coefficient of 0.989 in a wide range of food types, including cheese and other dairy products. Hayes *et al.*, 2001 obtained a correlation coefficient of 0.88 and the interception of regression line was 0.73 when they analyzed raw milk samples. In this work a high correlation coefficient and a linear regression interception closer to zero has been found.

Furthermore, Linton *et al.*, in 1997 reported that in conventional media certain mesophilic bacteria may be better recovered due to an optimal water activity and oxidation/reduction potential. In Crottin cheese these differences didn't affect the microbial counts. The lack of significant differences between means and the high correlation coefficient showed that petrifilm is a suitable and convenient alternative to the standard method for the enumeration of aerobic flora in Crottin cheese (De Sousa *et al.*, 2005).

The results of this study indicate that the Petrifilm method can replace the conventional pour plate method for the enumeration of hygiene indicator microorganisms in acidic fruit juices and that the pH of the juice sample will not affect the results. In addition, regardless of the method used, the counts of aerobic microorganisms in the juice samples were high: in 64.5% of the samples the counts were greater than 80 CFU/ml.

The results also indicates that freshly squeezed non-pasteurized juices regardless of the flavor, contained very high loads of microorganisms and should be of great concern to local health authorities. Pasteurized juices were found to be safer for human consumption than unpasteurized juices. HACCP guidelines should be made mandatory to all fresh juice manufacturers and vendors to follow in order to ensure that consumers are given an acceptable product in terms of the microbiological quality.

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