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Assessment of the Efficiency of Petrifim Method in Study of Bacteriological Quality of Some Homemade Dairy Products in Local Market of Basra

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Abstract
The aims of this study were assess the bacteriological quality of homemade milk products available in Basra city and to assess the performance of the Petrifilm method in the enumeration of microorganisms for dairy samples compared with conventional pour plating techniques. Comparisons for total aerobic plate count (APC), Lactic acid bacteria total coliform count and *Escherichia coli* count were done for 180 raw milk samples, 180 yogurt samples and 180 cheese samples. Samples were taken from local market of Basra during a 12-month project. This study has shown that these products have high levels of total aerobic bacteria, total coliform and *E. coli* which may be attributed to the absence of hygienic quality during production and storage because these products are usually produced by using traditional methods regardless of the quality of the raw milk used or the sanitary quality of the product. Significantly higher total coliform and *E. coli* were observed in raw milk, yogurt and cheese samples using Petrifilm compared to the pour plating technique ($P < 0.05$). However, no significant differences ($P > 0.05$) were found between APC pour plating method and Petrifilm during enumeration total aerobic plat count and lactic acid bacteria for all dairy samples. The study has shown that petrifilm is faster, more accurate and easier to use in counting microorganisms than conversional methods, which takes time to prepare and requires plates and other facilities. Therefore, we believe that the use of petrifilm methods in counting microorganisms for all kinds of foods is useful for saving time and effort compared to traditional methods.
Keywords: Petrifilm, Milk products, Lactic acid bacteria, Coliform, *E. coli*, Cheese

1.1 INTRODUCTION

Raw milk and its derivatives are considered as basic foods to all people; they are considered as an essential source for proteins, fat, sugar, minerals and vitamins which are required for the growth. Besides, dairy products form an important factor in the national economies of the producing countries (Robinson and Tamime, 2006). However, it has been detected that they also act as suitable medium carriers for the pathogenic and spoilage microorganism.

The microbiological quality of the raw milk used in producing other milk products is of the utmost importance for the safety of the product since it should be free of antibiotics and residues. The quantity of the morbid microbes should be within the standard accepted limits (total plate count less than10^4 CFU/mL) in addition to the absence of foodborne pathogenic bacteria (Bramley *et al*., 1990). Therefore, some traditional methods have been in use, such as plate count, to count all kinds of microorganisms. Recently, some new technologies were introduced, like the 3M™ Petrifilm™. These are dried ready-to-use plates which are like the agar of the petri dish, but they do not need any media preparation, are easy to use and do not require large laboratory and incubator spaces compared with standard pour plate procedure. They also contain enzymes that, by interacting with some materials from microorganisms, will produce multi-coloured bubbles to distinguish the different kinds of bacteria. This is done with the plantation of 1 ml of isolation sample of petrifilm, according to the set standards of AOAC (Linton *et al.*1997; Pattison *et al.*1998).

Despite the availability of numerous types of commercial dairy products, local soft white cheese and yogurt are still very popular in Iraq. Domestic yogurt and cheeses are usually produced from raw milk with deficient sanitary quality. Moreover, these products are manufactured and stored in poor hygienic environments. These dairy products may be contaminated at different times of manufacturing, handling and marketing. Therefore, this study has two objectives. The first objective is to assess the bacteriological quality of some homemade milk products available in Basra during all months of the year. The second objective is to assess the performance of Petrifilm method in the detection and enumeration of the bacteriological quality of milk products compared with the traditional methods of counting various microorganisms.

2. MATERIALS AND METHODS

2.1 Sample collection:

A total of 540 samples of homemade yogurt and cheese and cow raw milk was purchased randomly monthly from the local market of Basra, Iraq. Samples were transferred aseptically to a sterile plastic bag, and transported to the laboratory (n = 180 Raw milk, n = 180 Cheese, n = 180 Yogurt). Monthly, 45 samples were collected from 3 different locations in Basra between November 2013 and October 2014.

2.2 Determinations of Standard pour plate method:
For the enumeration of total viable counts, total coliforms and *E. coli* and lactic acid bacteria by Standard pour plate method 5g of each dairy sample (yogurt, cheese and 5 mL from raw milk) were mixed with 45 ml of Ringer solution (Oxoid), then Serial dilutions were prepared from homogenization samples. The pour plating method was applied to enumerate the various bacterial groups by placing 1 mL of each of the diluted samples on a petri dish and approximately 20 mL of melted selective medium was poured onto the plate. Plate Count Agar (APC, Oxoide) was used for enumerating aerobic plate count; the plates were incubated at 37 °C for 48 h (Harrigan and McCance, 1976). To enumerate coliforms and *E. coli*, Violet Red Bile Agar (VRBA, Oxoide) and Eosin Methylene Blue Agar (EMB, Oxoide) Agar medium were used. For coliforms enumeration the plates were incubated at 37 °C for 48 h while the plates carrying *E. coli* were incubated at 44°C for 24 and 48 (Harrigan and McCance, 1976). For enumerating Lactic acid bacteria (LAB) serial decimal dilutions were pour plated in de Man, Rogosa and Sharpe medium (MRS agar, Oxoide) and incubated at 37°C for 72 h under anaerobic conditions (Harrigan and McCance, 1976).

2.3 Petrifilm method:
3M™ Petrifilm™ Aerobic Count Plates AC (3M Microbiology, USA) were used for the enumeration of both total aerobic plate count and lactic acid bacteria, while 3M™ Petrifilm™ Coliform Count Plates (3M Microbiology, USA) were used for enumerating coliform and *E. coli* One milliliter of each of the diluted samples was placed on to Petrifilm™ Plates and incubated at 37°C for 24 h for coliform and *E. coli* Petrifilm™ Aerobic Count Plates were incubated for 24 - 48 h (Blackburn *et al.* 1996). To enumerate Lactic acid bacteria, dairy products samples were diluted in MRS broth then plated on Petrifilm™ AC (3M Microbiology. The plates were incubated at 37 °C for 24, 48 and 72 h under anaerobic conditions (GasPak EZ ™, BD).

2.4 Statistical analysis:
F test analysis (ANOVA) was used for statistical analysis of the data by using SPSS program (Release 18.0, SSPS Inc, Illinois, USA) then the least differences (LSD) were used to compare the means values.

3. RESULTS

3.1 Enumeration of aerobic Plate Count (APC) in milk products:
The total of aerobic plate counts in milk products (raw milk, yogurt, and cheese) was shown in figures (1, 2, 3). The total aerobic count (APC) range in raw milk was 3.67 - 5.67 $\log_{10}$ CFU/mL on Petrifilm™ AC and $\log_{10}$ CFU/mL 3.56 – 5.73 on pour plate counts (PCA) (Figure 1). In yogurt samples the counts of (APC) bacteria were detected to be between $\log_{10}$ CFU/mL 8.34 – 9.88 and $\log_{10}$ CFU/mL 8.12 – 9.95 on Petrifilm™ AC and pour plate counts (PCA) methods, respectively (Figure 2). Figure 3 shows that the total aerobic count (APC) range in cheese samples was 7.13 – 8.33 $\log_{10}$ CFU/mL on Petrifilm™ AC and $\log_{10}$ CFU/mL 7.09 – 8.33 on pour plate counts (PCA). The results showed that the number of total aerobic count (APC) in all tested samples of dairy products was significantly ($p< 0.05$) higher in summer months (May, June, July, August and September), compared with other months. Although these results indicated that the numbers of aerobic bacteria in all dairy products were different with the use of Petrifilm™ AC plates in comparison to the use of the pour plate counts during the period of the study which extended over the months of the year, ANOVA analysis indicated no significant differences ($P > 0.05$) between both methods.

3.2 Enumeration of Lactic acid bacteria (LAB) in milk products:
The total of Lactic acid bacteria (LAB) counts in milk products (raw milk, yogurt, and cheese) were shown in figures (4, 5, 6). Significantly greater ($P<0.05$) populations of the total of Lactic acid bacteria (LAB) were seen in summer months compared with cold months in all dairy products samples. The results showed that the highest numbers of Lactic acid bacteria (LAB) in raw milk was 4.32 $\log_{10}$ CFU/mL on Petrifilm™ AC and $\log_{10}$ CFU/mL 4.52 on conventional pour plating method (MRS) in July (Figure 4). Figure 5 shows that the highest numbers of Lactic acid bacteria (LAB) in yogurt samples was 9.09 $\log_{10}$ CFU/mL on Petrifilm™ AC and $\log_{10}$ CFU/mL 9.22 on conventional pour plating method (MRS). In cheese samples the highest counts of the detected Lactic acid bacteria (LAB) was 5.41 $\log_{10}$ CFU/mL and 5.54 $\log_{10}$ CFU/mL on Petrifilm™ AC in May. There was no significant difference ($P > 0.05$) in lactic acid bacteria counts between Petrifilm™ AC and conventional pour plating method over the months of the year.

3.3 Enumeration of total coliform and total E. coli in milk products:
Table 1 shows a variation in the logarithm of the number of coliform in dairy samples (raw milk, yogurt and cheese). The results showed that the number of coliform in all tested samples of dairy products were significantly ($p< 0.05$) higher in summer months compared with other months. The counts of coliform were detected to be between 0 – 2.41 $\log_{10}$ CFU/mL on Petrifilm™ and 0 - 1.77 on conventional methods (VRB ager), 0 – 3.99 $\log_{10}$

CFU/mL on Petrifilm™ and 0 – 3.48 on conventional methods (VRB ager) and 1.20 – 4.92 $\log_{10}$ CFU/mL on Petrifilm™ and 0 – 4.67 on conventional methods (VRB ager) in dairy samples (raw milk, yogurt and cheese), respectively. Thus, coliform counts were significantly higher ($P > 0.05$) on Petrifilm™ method compared with conventional methods (VRB ager) over the months of the year.

The number *E. coli* in dairy samples (raw milk, yogurt and cheese) was shown in Table 2. The results show that the numbers of *E. coli* in all samples of dairy products were significantly ($p< 0.05$) higher in summer months compared with other months. The count of *E. coli* range was 0 – 1.99 $\log_{10}$ CFU/mL on Petrifilm™ and 0 - 1.67 on conventional methods (EMB ager), 0 – 2.33 $\log_{10}$ CFU/mL on Petrifilm™ and 0 – 2.07 on conventional methods (EMB ager) and 0 – 3.60 $\log_{10}$ CFU/mL on Petrifilm™ and 0 – 3.07 on conventional methods (EMB ager) in dairy samples of raw milk, yogurt and cheese, respectively. The results indicated that *E. coli* counts were significantly higher ($P > 0.05$) on Petrifilm™ method compared with conventional methods (EMB ager) over the months of the year.

4. DISCUSSION

The total aerobic plate count test is a useful indicator for determining the hygienic conditions present during the manufacturing and processing of raw milk by plating on plate count ager and incubation in aerobic conditions (Chambers, 2002). In this study it was found that the mean number of total aerobic counts (APC) of raw milk tested samples was higher than 10^5 CFU/ml in most summer months (from June to October). This high count of APC may be due to unhygienic conditions during production, collection and transport. Another reason for the high bacterial load for raw milk can be the contamination of this milk with mastitis milk (Chambers, 2002). The raw milk drawn from a healthy cow usually contains a low bacterial load (less than 10^3 CFU/mL), but the loads may increase up to 10^5 CFU/mL if it is kept for some time at room temperatures (Richter *et al*., 1992). The results of this study also show that high numbers of the aerobic bacteria was found in local Iraqi white soft cheese samples. Many types of microorganisms may enter the cheese during production, handling and marketing because local Iraqi white soft cheese is usually produced under unhygienic conditions. The high APC numbers of cheese samples may be attributed to the growth of bacteria during different stages of production, which might be explained by the initial bacterial counts of the raw milk. In this study no significant ($P < 0.05$) was found between Petrifilm™ AC and APC ager for the enumeration of total aerobic plate count in all dairy products samples. Similar findings have been reported by previous studies on comparative assessments between Petrifilm™ and conventional methods to determine the microbial quality of different foods, no significant differences between the 3 M™ Petrifilm™ AC plate counts and the pour plate counts were obtained during enumeration of the total aerobic counts in goat soft cheese and in fermented chili mashes (De Sousa *et al.,* 2005, Beall *et al.* 2012). The results showed that Lactic acid bacteria were a main part of the total bacteria in raw milk, the high numbers of LAB may cause defective fermentative acidification of raw milk. So, efficient procedures should be taken to avoid this type of spoilage. According to the obtained results, non-significant difference was observed between Petrifilm™ AC and conventional pour plating method (MRS) for the enumeration of lactic acid bacteria in all dairy products. These results are consistent with observations made by previous studies, no significant difference between Petrifilm plates and convectional method (M17 agar) was found during the enumeration of lactococci counts in milk (Champagne *et al.* 1994). Also, no significant difference ($P < 0.05$) and a high correlation index between Petrifilm™ AC and MRS ager was observed for the enumeration of lactic acid bacteria in fermented milk products products (Nero *et al.* 2006, Nero *et al.* 2008). Other study has also reported similar finding, when using Petrifilm AC plates to enumerate lactic acid bacteria cultures in frozen yogurt mix (McGregor *et al.*, 1995).

The coliform test has been used for the assessment of the effectiveness of sanitary systems during milking and milk products production. Coliforms in milk and milk products are suggestive of unhygienic conditions or practices during manufacturing and processing. *Escherichia coli* is considered as a faecal indicator bacterium, whose recovery from milk and milk products is an indicator of the presence of other organisms that may be pathogenic bacteria (Christen *et al.* 1992). The results of this study reported that high contamination with total coliform bacteria and *E. coli* was recorded in all analyzed dairy products during summer months from June to October. The improper bacteriological content was related to poor hygiene standards in the manufacturing process and transportation of these products. This might be attributed to the variation of temperature throughout the months of the year; it has been observed that the logarithm of the number of bacteria increased during the hot months of summer and the moderate heat of spring, but it considerably decreased during the cold months of winter. This is due to the capability of bacteria to adapt with the surrounding environment. These results are consistent with observations made by Arabi *et al*. (2014) conducted on camel meat, which showed the difference between the numbers of coliform and *E. coli* with a considerable decrease during the cold winter. Also, the numbers of coliform and *E. coli* decrease during the cold months and they increase during the hot and moderate months of the year in minced beef sold in market Basra city (George *et al*., 2016). The Coliform bacteria constitute the most risky group among bacteria that contaminate cheeses through several sources (Giammanco *et al*., 2011). These microorganisms may be infect milk through the milker, cow skin and the air in the shed. The incidence of a high numbers of *E. coli*

in raw milk and homemade Iraqi white cheese and yogurt samples may be due to the absence of suitable sanitation and insufficient heating of milk use for cheese or yogurt production. The count of total coliform bacteria and *E. coli* are found to be high particularly in soft white cheeses that are manufactured particularly at small family enterprises under simple conditions and that are presented for sale in open form in local markets.

Therefore, stringent hygienic processes must be followed and pasteurization of milk should be imposed to avoid contamination of cheese and yogurt with coliforms and *E. coli*. Several diarrheal diseases caused by ingestion of contaminated milk and milk products with *E. coli* bacteria. It was reported that 1 - 5% of foodborne illnesses were related to the consumption of milk and milk products and that 53% of cases of food-borne illnesses are caused by contaminated cheese (Schrade and Yager, 2001).

As for the enumeration of total coliforms and *E. coli* by Petrifilm™ and conventional methods, the results show that the numbers of these bacteria remained almost similar in both methods in all dairy products. Therefore, Petrifilm™ offers a good alternative for the enumeration and detection of microorganisms in dairy products because it is suitable and gives good results. Petrifilm™ method combined with selective culture media (conventional methods) for enumeration of coliform and *E. coli* has been described in similar studies, it was indicated that the use of Petrifilm™ as an alternative method for the enumeration of coliform and *E. coli* is gaining international acceptance due to its practicality, being faster, more accurate and easier to use than plate counts, in addition to efficiency of this method in the detection of fecal coliform contamination through *E. coli* direct identification (De Sousa *et al.* 2015). Also, Carvalho *et al*. (2002) suggested to use of Petrifilm™ as an alternative to the conventional methods of total coliforms count in refrigerated raw milk. Moreover, no any difference was demonstrated between conventional methods and Petrifilm method during enumeration total coliform and *E. coli* in sheep milk (De Sousa *et al.* 2015).

5. CONCLUSION

In Iraq, milk and its products are produced at homes or in small mills, and are highly consumed by individuals. During study and follow the sanitary quality of milk and homemade dairy products (cheese, yogurt) sold to the general people in Basra city in all months of the year. It was found that some of these homemade dairy products are of high unsanitary quality since they contain high numbers of microorganism. These products are quickly spoiled and form a suitable environment for the culturing of microbes that transfer diseases to the human body, like food poisoning. So it is very crucial to keep with the sanitary standards during the production, marketing and storing phases. A comparison between Petrifilm method and conventional methods for detection of total aerobic plat count and lactic acid bacteria in these dairy products. No significant differences were obtained between both methods. While, The results indicate that the Petrifilm method used in this study is better for recovery of coliform and *E. coli* from dairy samples than conventional methods.

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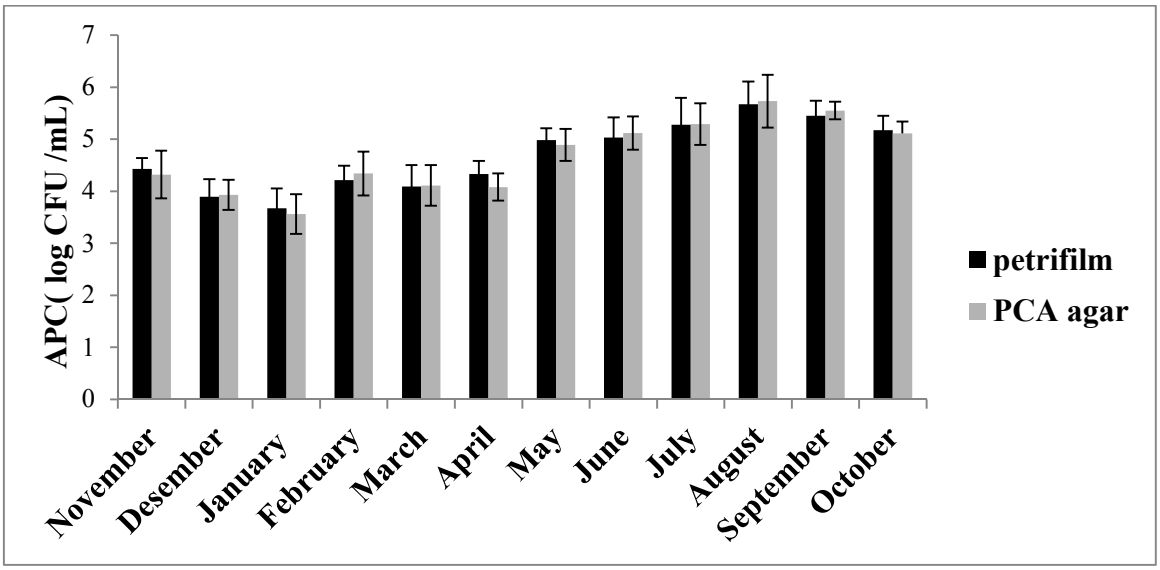

Figure 1. Counts of aerobic plate counts (APC) in PCA ager and 3M™ Petrifilm™ Aerobic Count Plate (AC) from raw milk samples. Values represent means ±SEM (n=3).

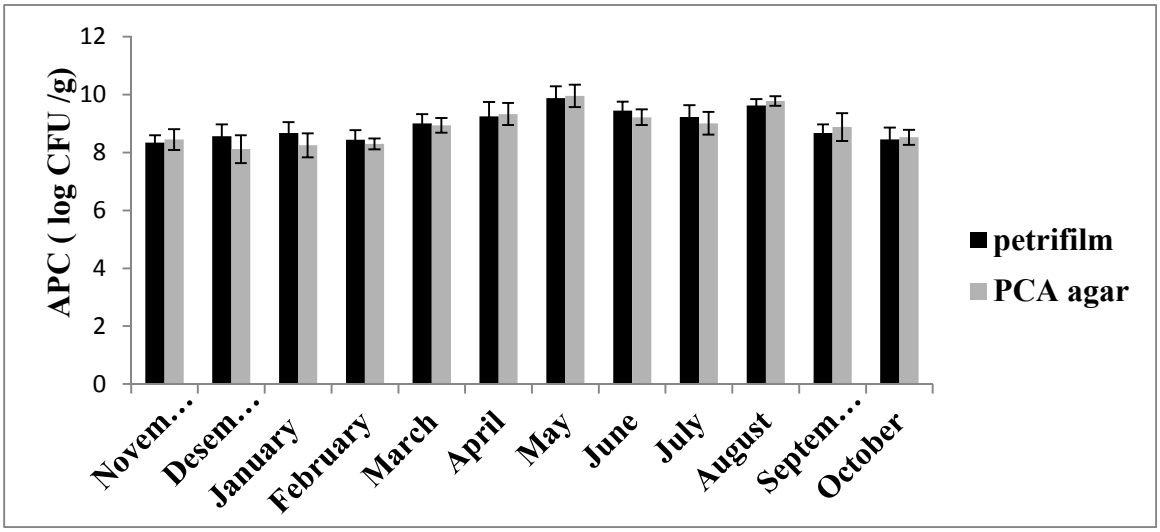

Figure 2. Counts of aerobic plate counts (APC) in PCA ager and 3M™ Petrifilm™ Aerobic Count Plate (AC) from Yogurt samples. Values represent means ±SEM (n=3).

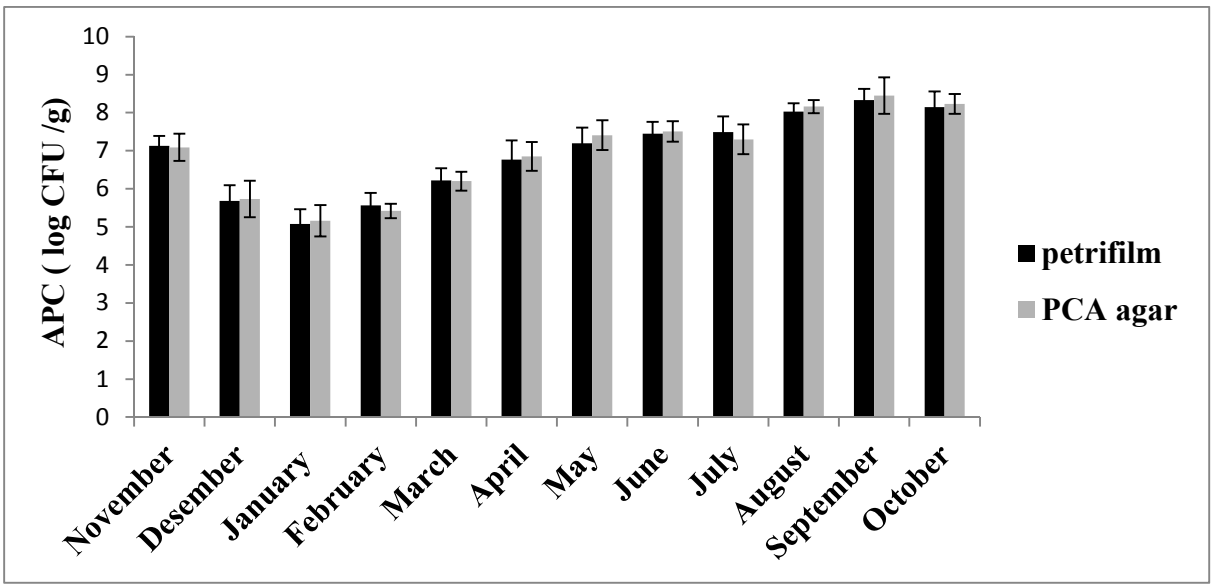

Figure 3. Counts of aerobic plate counts (APC) in PCA ager and 3M™ Petrifilm™ Aerobic Count Plate (AC) from Cheese samples. Values represent means ±SEM (n=3).

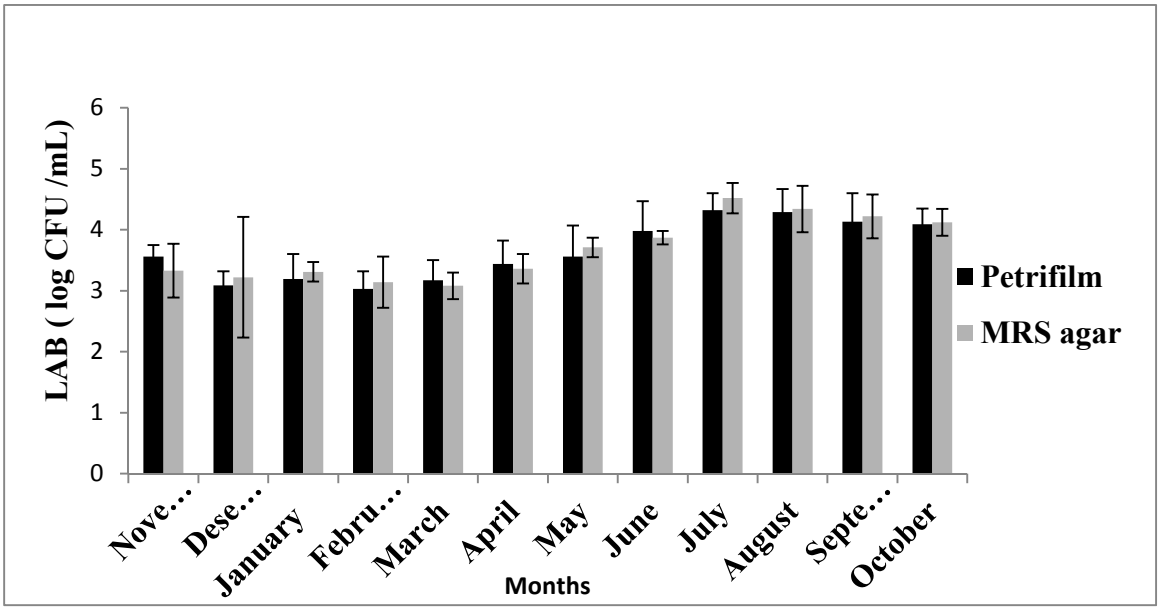

Figure 4. Counts of lactic acid bacteria (LAB) in MRS ager and 3M™ Petrifilm™ Aerobic Count Plate (AC) from raw milk samples. Values represent means ±SEM (n=3).

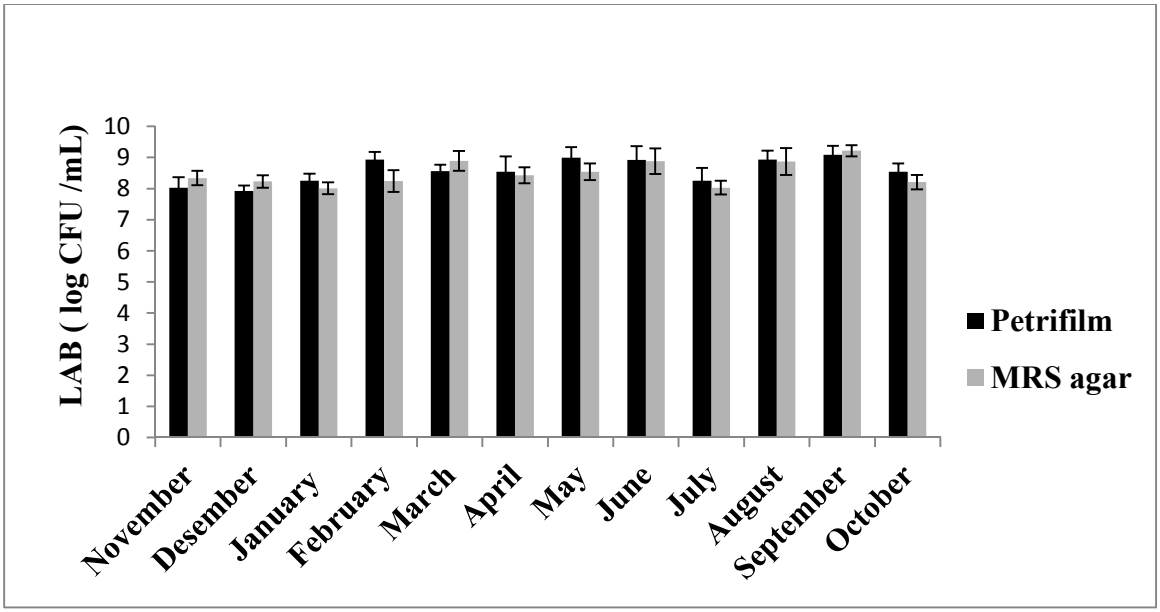

Figure 5. Counts of lactic acid bacteria (LAB) in MRS ager and 3M™ Petrifilm™ Aerobic Count Plate (AC) from Yogurt samples. Values represent means ±SEM (n=3).

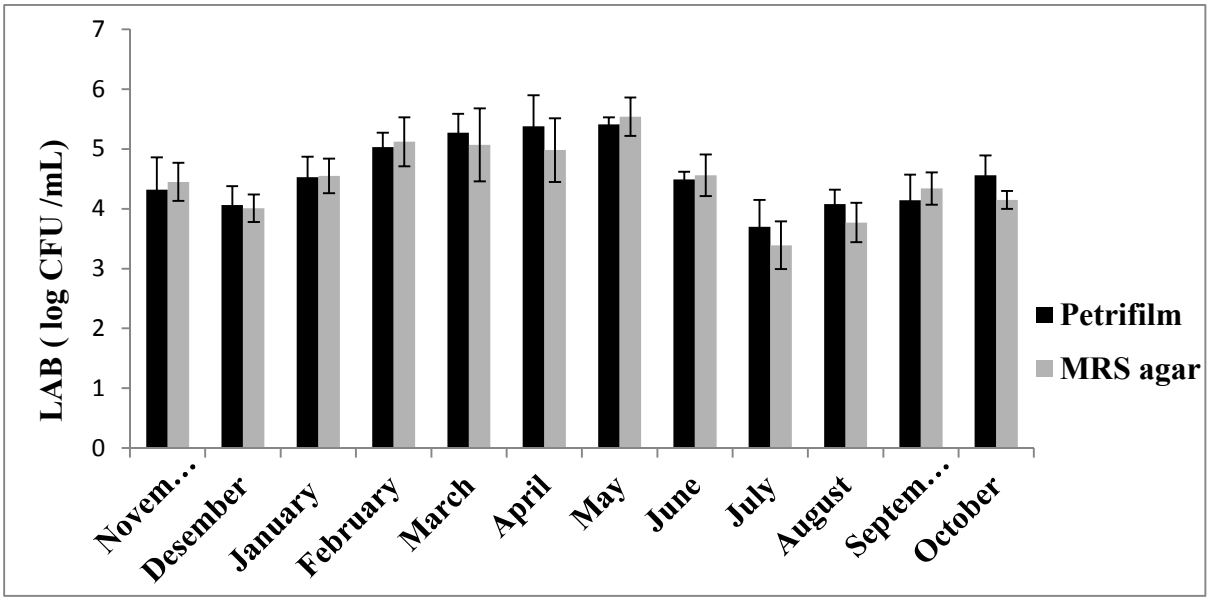

Figure 6. Counts of lactic acid bacteria (LAB) in MRS ager and 3M™ Petrifilm™ Aerobic Count Plate (AC) from Cheese samples. Values represent means ±SEM (n=3).

Table 1. Counts of total Coliform in violet red bile ager (VRB) and 3M™ Petrifilm™ *E*. coli/Coliform Count Plates from dairy samples (A= raw milk, B = Yogurt and C = cheese). Values represent means ±SEM (n=3).

Sample date	Raw milk		Yogurt		Cheese	
	VRB ager	Petrifilm	VRB ager	Petrifilm	VRB ager	Petrifilm
November	1.11 ± 0.12	1.38± 0.20	1.71 ± 0.09	1.96 ± 0.10	2.03 ± 0.13	2.31 ± 0.09
December	0	1.42 ± 0.10	0	0	1.29 ± 0.08	1.57 ± 0.12
January	0	0	0	0	0	1.20 ± 0.03
February	0	0	0	0	1.02 ± 0.11	1.31 ± 0.14
March	1.2 ± 0.12	1.47 ± 0.15	1.39 ± 0.13	1.69 ± 0.90	1.51 ± 0.10	1.96 ± 0.09
April	0	1.41 ± 0.10	2.72 ± 0.15	2.89 ± 0.12	1.49 ± 0.06	1.88 ± 0.02
May	1.32 ± 0.19	1.65 ± 0.11	2.60 ± 0.14	2.77 ± 0.19	2.18 ± 0.08	2.43 ± 0.12
June	1.52 ± 0.13	1.89 ± 0.09	1.73 ± 0.11	1.96 ± 0.12	3.06 ± 0.14	3.22 ± 0.08
July	1.46 ± 0.14	1.93 ± 0.14	2.42 ± 0.07	2.65 ± 0.08	4.67 ± 0.09	4.92 ± 0.04
August	1.72 ± 0.15	2.17 ± 0.11	3.48 ± 0.10	3.99 ± 0.09	4.51± 0.085	4.89 ± 0.23
September	1.77 ± 0.16	2.34 ± 0.12	2.02 ± 0.12	2.32 ± 0.18	3.67 ± 0.12	4.22 ± 0.12
October	1.69 ± 0.18	2.41 ± 0.16	1.71 ± 0.11	1.96 ± 0.20	3.66 ± 0.13	4.05 ± 0.11

Table 2. Counts of *E*.coli in Eosin methylene blue (EMB) and 3M™ Petrifilm™ *E*. coli/Coliform Count Plates from dairy samples (raw milk, Yogurt and cheese). Values represent means ±SEM (n=3).

Sample date	Raw milk		Yogurt		Cheese	
	EMB ager	Petrifilm	EMB ager	Petrifilm	EMB ager	Petrifilm
November	0	1.22 ± 0.14	1.20 ± 0.08	1.42 ± 0.12	1.31 ± 0.34	1.82 ± 0.08
December	0	1.03 ± 0.31	0	0	0	1.01 ± 0.28
January	0	0	0	0	0	0
February	0	0	0	0	0	0.69 ± 0.16
March	1.01 ± 0.13	1.31 ± 0.11	0	1.02 ± 0.12	0	1.32 ± 0.18
April	0	1.17 ± 0.25	1.29 ± 0.23	1.95 ± 0.18	1.01 ± 0.23	1.38 ± 0.20
May	1.23 ± 0.18	1.51 ± 0.20	2.07 ± 0.16	2.18 ± 0.14	1.08 ± 0.19	1.43 ± 0.10
June	1.30 ± 0.16	1.62 ± 0.19	1.37 ± 0.12	1.52 ± 0.09	2.01 ± 0.23	2.37 ± 0.17
July	1.32 ± 0.12	1.71 ± 0.15	1.94 ± 0.19	2.03 ± 0.12	2.53 ± 0.19	3.19 ± 0.13
August	1.41 ± 0.17	1.68 ± 0.23	2.04 ± 0.25	2.19 ± 0.23	3.08 ± 0.07	3.60 ± 0.21
September	1.44 ± 0.23	1.82 ± 0.14	1.06 ± 0.11	1.33 ± 0.15	2.33 ± 0.15	3.10 ± 0.31
October	1.67 ± 0.15	1.99 ± 0.13	1.01 ± 0.30	1.26 ± 0.20	2.12 ± 0.13	2.55 ± 0.15