

Evaluation of Petrifilm™ method for enumerating aerobic bacteria in Crottin goat's cheese

G.B. de SOUSA¹, L.M. TAMAGNINI^{1*}, R.D. GONZÁLEZ¹, C.E. BUDDÉ².

¹ *Cátedra de Microbiología, Departamento de Fisiología, Facultad de Ciencias Exactas, Físicas y Naturales, Av. Vélez Sársfield 299, X5000JJC. Córdoba, Argentina*

² *Facultad de Matemática, Astronomía y Física, Ciudad Universitaria, 5000 Córdoba, Argentina.*

*Correspondence. E-mail: ltamagnini@com.uncor.edu

SUMMARY

The Petrifilm™ Aerobic Count Plate (ACP) developed by 3M laboratories, is a ready-to-use culture medium system, useful for the enumeration of aerobic bacteria in food. Petrifilm™ was compared with a standard method in several different food products with satisfactory results. However, many studies showed that bacterial counts in Petrifilm™ were significantly lower than those obtained with conventional methods in fermented food. The purpose of this study was to compare the Petrifilm™ method for enumerating aerobic bacteria with a conventional method (PCA) in Crottin goat's cheese. Thirty samples were used for the colony count. The mean count and standard deviation were $7.18 \pm 1.17 \log \text{CFU g}^{-1}$ on PCA and $7.11 \pm 1.05 \log \text{CFU g}^{-1}$ on Petrifilm™. Analysis of variance revealed no significant differences between both methods ($t = 1.33$, $P = 0.193$). The Pearson correlation coefficient (0.971 , $P=0.0001$) indicated a strong linear relationship between the Petrifilm™ and the standard method. The results showed that Petrifilm™ is suitable and a convenient alternative to this standard method for the enumeration of aerobic flora in goat soft cheese.

Keywords: Goat's cheese, total bacterial count, Petrifilm™

RESUMEN

Evaluación de Petrifilm™ para la enumeración de bacterias aerobias en queso de cabra Crottin. Petrifilm™ Aerobic Count Plate (ACP) desarrollado por 3M es un sistema listo para usar, empleado para el recuento de bacterias aerobias en alimentos. Petrifilm™ fue comparado con los métodos estándar en diferentes productos alimenticios con resultados satisfactorios. Sin embargo, en alimentos fermentados, algunos estudios mostraron que el recuento de bacterias aerobias en Petrifilm™ fue significativamente menor que aquellos obtenidos con los métodos convencionales (PCA). El propósito de este estudio fue comparar el método Petrifilm™ para el recuento de bacterias aerobias con un método convencional en queso de cabra Crottin. Se usaron 30 muestras para el recuento de colonias. Las medias y desviaciones estándar fueron $7,18 \pm 1,17 \log \text{UFC g}^{-1}$ en PCA y $7,11 \pm 1,05 \log \text{UFC g}^{-1}$ en Petrifilm™. El análisis de varianza mostró que no había diferencia significativa entre ambos métodos ($t = 1,33$, $P = 0,193$). El coeficiente de correlación fue $0,971$ ($P = 0,0001$) indicando una fuerte correlación lineal. Los resultados muestran a Petrifilm™ como un método apropiado y una alternativa conveniente a los métodos estándar para la cuantificación de flora aeróbica en queso blando de cabra.

Palabras clave: queso de cabra, recuento aeróbico total, Petrifilm™

The Petrifilm™ Aerobic Count Plate (ACP) developed by 3M laboratories, is a ready-to-use culture medium system, useful for the enumeration of aerobic bacteria in food. It has been validated as an alternative for aerobic count by international associations such as American Public Health Association (APHA, 1992) (1).

Conventional techniques for the enumeration of microorganisms in food are laborious and material-intensive. Petrifilm™ ACP system contains standard nutrients, a cold-water-soluble jelling agent and a tetrazolium indicator that eases and speeds colony enumeration. Petrifilm™ ACP plates are ready to receive 1.0 ml of sample with the benefits of saving time, labour and the space that an incubator requires.

Petrifilm™ ACP was compared with the standard method for the enumeration of aerobic flora in several different food products with satisfactory results. (3, 6, 9,12). However, some workers have found differences in the count between both methods in some fermented foods (14).

The application of this system, as regards the quality control in goat cheese, might be of great interest to the dairy farmer. Goat's milk has many differences from cow's and ewe's milk, which influence the flavor and taste of dairy products such as cheese. In the developed countries goat's milk production recorded an upturn in recent years, and 95% of this milk is transformed into typical dairy products, mostly because of an origin and quality connotation along the region (4).

The aim of this work was to compare the Petrifilm™ ACP method with Aerobic Plate Count for enumerating aerobic bacteria in Crottin goat's cheese.

Crottin is a mould-ripened soft lactic cheese made "on farm" with goat's milk heat-treated at 70 °C for 30 min. Liquid rennet, starter culture, CaCl₂, and *Penicillium candidum* are added during the elaboration procedure. It is cold ripened (8-12 °C) during 15 days. The shelf life is 45 days. Thirty units made on farm (Córdoba, Argentina), with different ripening time were carried to the laboratory in a cold container (2 °C - 6 °C) and analysed within 6 h.

Representative 5 g cheese samples were homogenized with 45 ml sterile 0.1% peptone water with 0.1% Tween 80 for 2 min, then serial 10- fold dilutions were prepared and plated in duplicate (1). Inoculated Petrifilm™ plates and standard pour plates were incubated at 30 °C for 48 h for its count.

For the statistical analysis the count of Colony Forming Units (CFU) was transformed to log CFU g⁻¹. Paired *t*-test, Pearson correlation coefficient and linear least square regression were used to compare both methods. All statistical analysis were performed with SigmaStat 2.03 software (SPSS Inc.)

The Petrifilm™ has the advantage of being easy to use, traditional media preparation is unnecessary, and saves both labour and time. On the other hand, the only disadvantages seen in this work was that on overcrowded plates the center didn't show visible colonies and many small colonies were seen on the edges. When this occurred, further dilution of the sample was required.

The mesophilic aerobic count (MAC) range was 5.11 – 9.20 log CFU g⁻¹ on PCA and 5.53 – 9.00 log CFU g⁻¹ on Petrifilm™. The wide count range could be influenced by the different ripening times of some cheeses. The mean count and standard deviation were 7.18 ± 1.17 log CFU g⁻¹ on PCA and 7.11 ± 1.05 on Petrifilm™. The MAC was higher than those obtained in a previous study on Crottin cheese with 15 ripening days (5.89 ± 0.49 log CFU g⁻¹) (15). Park *et al.* (2004) (13) obtained lower total plate count in soft goat cheese on Petrifilm™ (5.58 log CFU g⁻¹). Variable numbers of MAC were reported for Anevato and Brie cheeses: 8.08 to 8.70 log CFU g⁻¹ (7) and 6.50 log CFU g⁻¹ (11) respectively.

Analysis of variance revealed no significant differences between both media (*t* = 1.33, *P* = 0.193). Wide ranges and standard deviations higher than 1 log unit were probably due to the difference in the ripening times of the cheeses. The Pearson correlation coefficient (0.971, *P* = 0.0001) indicated a strong linear relationship between the Petrifilm™ and the standard methods (Fig. 1). The regression analysis yielded the equation:

$$\log \text{PCA} = -0.567 + 1.09 \log \text{Petrifilm}^{\text{TM}}$$

Previous studies on comparative evaluations between Petrifilm™ and conventional methods to determine the

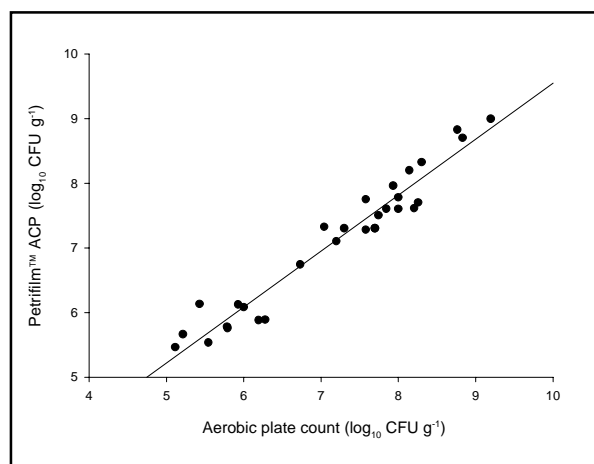


Figure 1. Linear regression of aerobic plate count (log₁₀ CFU g⁻¹) versus Petrifilm™ ACP (log₁₀ CFU g⁻¹) in Crottin cheese.

microbial quality of different foods showed remarkable correlation between the two methods, *r* = 0.989 (2, 5), *r* = 0.97 (12). Blackburn *et al.*, (1996) (3) found a correlation coefficient of 0.989 in a wide range of food types, including cheese and other dairy products. Hayes *et al.*, (2001) (8) obtained a correlation coefficient of 0.88 and the interception of regression line was 0.73 when they analysed raw milk samples. In this work a high correlation coefficient and a linear regression interception closer to zero has been found. However, Pattison *et al.* (1998) (14), showed that bacterial counts in Petrifilm™ were significantly lower than those obtained with conventional methods (5.34 log CFU g⁻¹ and 5.96 log CFU g⁻¹, respectively) in sorghum beer. The majority (88%) of the colonies isolated from both methods were lactic acid bacteria (LAB). These authors speculated that differences in the composition of the conventional media and the corresponding Petrifilm™ may have resulted in different bacterial counts. They recommended taking in consideration these statements when Petrifilm™ is used for the routine enumerations of products containing predominantly lactic acid bacteria, for example cultured dairy products. Furthermore, Linton *et al.*, (1997) (10) 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.

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REFERENCES

1. APHA (1992) In: Vanderzant, C and Splittstoesser, DF (Eds.). Compendium of Standard Methods for the Microbiological Examination of Food. APHA (American Public Health Association) Inc. Washington, DC, USA.
2. Beuchat LR, Copeland F, Curiale MS, Danisavich T, Gangar V, King BW, *et al.* (1998) Comparison of the SimPlate™ Total Plate Count method with Petrifilm™, Redigel™ and Conventional pour-plate methods for enumerating aerobic microorganisms in foods. *J. Food Prot.* 61: 14-18.
3. Blackburn C de W, Baylis CL, Pettitt SB (1996) Evaluation of Petrifilm™ methods for enumeration of aerobic flora and coliforms in wide range of foods. *Lett. Appl. Microbiol.* 22: 137-140.
4. Boyazoglu J, Morand-Fehr P (2001) Mediterranean dairy sheep and goat products and their quality A critical review. *Small Rumin. Res.* 40: 1-11.
5. Chain VS, Fung DYC (1991) Comparison of Redigel, Petrifilm™, Spiral Plate System Isogrid and Aerobic Plate Count Agar for determining the numbers of aerobic bacteria in selected foods. *J. Food Prot.* 54: 208-211.
6. Ellis P, Meldrum R (2002) Comparison of the compact dry TC and 3M Petrifilm ACP dry sheet media methods with the spiral plate method for the examination of randomly selected foods for obtaining aerobic colony counts. *J. Food Prot.* 65: 423-425.
7. Hatzikamari M, Litopoulou-Tzanetaki E, Tzanetakis N (1999) Microbiological characteristics of Anevato: a traditional Greek cheese. *J. Appl. Microbiol.* 87: 595-601.
8. Hayes MC, Ralyea RD, Murphy SC, Carey NR, Scarlett JM, Boor KJ (2001) Identification and characterization of elevated microbial counts in bulk tank raw milk. *J. Dairy Sci.* 84: 292- 298.
9. Jordano R, Lopez C, Rodriguez V, Cordoba G, Medina LM, Barrios J (1995) Comparison of Petrifilm method to conventional methods for enumerating aerobic bacteria, coliforms, *Escherichia coli* and yeast and molds in foods. *Acta Microbiol. Immunol. Hung.* 42: 255-259.
10. Linton RH, Eisel WG, Muriana PM (1997) Comparison of conventional plating methods and Petrifilm for the recovery of microorganisms in a ground beef facility. *J. Food Prot.* 60: 1084-1088.
11. Little CL, Knochel S (1994) Growth and survival of *Yersinia enterocolitica*, *Salmonella* and *Bacillus cereus* in Brie stored at 4, 8, and 20 °C. *Int. J. Food Microbiol.* 24: 137-145.
12. Mizuochi S, Kodaka H (2000) Evaluation of dry sheet medium culture plate (compactdry TC) method for determining numbers of bacteria in food samples. *J. Food Prot.* 63: 665-667.
13. Park YW, Kalantari A, Frank JF (2004) Changes in the microflora of commercial soft goat milk cheese during refrigerated and frozen-storage. *Small Rumin. Res.* 53: 61-66.
14. Pattison TL, Geornaras I, von Holy A (1998) Microbial populations associated with commercially produced South African sorghum by conventional and Petrifilm™ plating. *Int. J. Food Microbiol.* 43: 115-122.
15. Tamagnini LM, de Sousa GB, González RD, Revelli J, Budde CE (2005) Behavior of *Yersinia enterocolitica* and *Salmonella typhimurium* in Crottin goat's cheese. *Int. J. Food Microbiol.* 99: 129-134.