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SHORT PAPER

Comparison of 3M-Petrifilm™ and conventional methods for quick counting of aerobics on confectionery products

Comparación de métodos 3M-Petrifilm TM y convencional para conteo rápido de aerobios en productos de confitería

Fernando Coronado De La O[a*]

[a] *Universidad Juárez Autónoma de Tabasco, Av. Universidad s/n, Zona de la Cultura, Col. Magisterial, C.P. 86040, Centro, Tabasco, México.*

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[*] *Corresponding author:*
Fernando Coronado De La O
Phone: +52 (993) 358 15 00
Electronic mail address: fercdelao@ujat.mx

A B S T R A C T

As a requirement to evaluate the quality of food products, the counting of aerobic mesophilic microorganism is used. Effectiveness of applying a fast methodology in order to detect the existence of these microorganisms is crucial to take corrective actions in processing facilities. This study aimed to compare the alternative method 2015.13 AOAC vs the traditional method based on tryptone soy agar for aerobes fast counting confectionery products. The matrix chocolate coating was internally inoculated with *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. The matrix cocoa powder showed natural contamination. 5 levels of inoculum were tested: 60, 380, 700, 36 000, 40 000 UFC/g. The culture temperature was about 35°±1°C during 24±2 hours for the method AOAC 2015.13 and 48±2 hours for the conventional method. Results demonstrated that there are no statistical difference between matrixes based on chocolate coatings and cocoa powder, on 3M-PetrifilmTM and tryptone soy agar plates, for aerobes fast counting with inocula 60 (p=0,506), 380 (p=0,843), 700 (p=0,378), 36 000 (p=0,180) and 40 000 (p=0,180), with a correlation coefficient of r=0.99 (p≤0.05), precision Rp%≤2.0*RDS%Poison and recovery ≥ 80%. The 3M-PetrifilmTM RAC Alternative Microbiological Counting Method has been shown to be reproducible, reliable, and accurate.

Keywords: Confectionery products, 3M-Petrifilm, counting of aerobics

R E S U M E N

Para evaluar la calidad de productos alimenticios se emplea como requisito de cumplimiento el recuento de aerobios mesófilos. La efectividad de aplicar una metodología rápida para detectar la presencia de estos microorganismos es fundamental para la toma de acciones correctivas en plantas procesadoras. El objetivo del estudio fue comparar el método alternativo 2015.13 AOAC con el método tradicional a base de agar triptona de soya para el conteo rápido de aerobios en productos de confitería. La matriz coberturas de chocolate fue inoculada internamente con *Escherichia coli* ATCC 25922 y *Staphylococcus aureus* ATCC 25923. La matriz polvo de cacao presentó contaminación natural. Se probaron 5 niveles de inóculo: 60, 380, 700, 36 000, 40 000 UFC/g. La temperatura de cultivo fue 35°±1°C durante 24±2 horas para el método AOAC 2015.13 y 48±2 horas para el método convencional. Los resultados evidenciaron que no hay diferencias significativas entre las matrices a base de cobertura de chocolate y polvo de cacao, en las placas 3M-PetrifilmTM y agar triptona de soya, para el recuento rápido de aerobios con los inóculos 60 (*p*=0,506), 380 (*p*=0,843), 700 (*p*=0,378), 36 000 (*p*=0,180) y 40 000 (*p*=0,180), con un coeficiente de correlación r=0.99 (p≤0.05), precisión Rp%≤2.0*RDS% Poison y recuperación ≥ 80%. Se evidenció que el método de conteo microbiológico alternativo 3M-PetrifilmTM RAC es reproducible, confiable y preciso.

Palabras clave: Productos de confiteria, 3M-Petrifilm, cuenta de aerobios en placa

INTRODUCTION

Mesophilic aerobic counting is frequently used in the food industry to measure the sanitary quality of food products throughout the production process, starting with raw materials used as ingredients to finished products. Although it is not used as an indicator of safety in food products, the mesophilic aerobic count is useful to provide information on the deficiencies of the sanitation systems, in the control systems and in the sanitary conditions of the storage and processing facilities (4). In the food industry, fast and accurate inspection is important as it must simultaneously consider both mass production and food quality concerns (11,12). For this, rapid microbiological techniques are a valid alternative. Most of these methods have attributes of conventional techniques (especially reliability) and in addition, the time between sampling and results is shorter (2,4). Among the conditions that rapid methods meet are their accuracy, speed, minimum cost, acceptability, ease of use, reliability of the method, adequate technical support and a minimum required useful space (5,8). Increased public awareness of food safety issues, coupled with increased government regulation of the food industry, has dramatically increased the amount and type of microbiological tests that are routinely performed in quality control laboratories (3). Reference methods for counting mesophilic aerobes indicate that there are responses between 48 and 72 hours, according to Benzinger *et al.* (2014)(2) and Bird *et al.* (2016)(4). The 3M Petrifilm™ Rapid Aerobic Count (RAC) Plates is a ready-to-use system that contains nutrients, a water-soluble gelling agent, and an indicator that facilitates colony enumeration (2,4). To assess the quality of cocoa powder and chocolates, the count of mesophilic aerobes is required as a compliance requirement (6-9). With this background, a study was planned that aimed to: Compare the alternative method 2015. AOAC with the traditional method based on tryptone soy agar, for the rapid count of aerobes, in matrices based on chocolate coatings and cocoa powder.

MATERIALS AND METHODS

The study was carried out in Food Microbiology Lab of Universidad Juárez Autónoma de Tabasco, Tabasco, Mexico. As experimental unit, 5 samples of cocoa-based products were used: three samples of cocoa powder, a sample of chocolate cream and a sample of chocolate coating. In the experimental units: cocoa powder and chocolate creams, the microbiological contamination was natural; and, in the case of chocolate coatings, it was artificially contaminated with strains of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 (1). They were evaluated following the method recommended by the supplier of the 3M-PetrifilmTM Plates. Subsequently following the conventional method, the cocoa product samples were homogenized, diluted and deposited (1 mL) in Petri dishes with tryptone soy agar (TSA, pH 7.3, Merck KGaA, Darmstadt, Germany), melted at 47- 50 °C, where the contents of the Petri dish were mixed using rotating movements (10). The colonies observed in TSA were incubated in a time of 48 ± 2 h at 35 °C. The results were reported as CFU mesophilic aerobic count for each gram of food. The count values obtained were transformed to log10 for both the 3MPetrifilmTM method and the conventional method. The linear regression method ($p \leq 0.05$) was used (9-12), whose mathematical model is: $Y = a + bX$

RESULTS AND DISCUSSION

Figure 1 shows the results of the linear regression in which the conventional method is related to the alternative 3M-Petrifilm™ RAC method. The regression equation was: Y=0.9983x-0.0114. Determination coefficient R^2= 0.99, correlation R = 0.995.

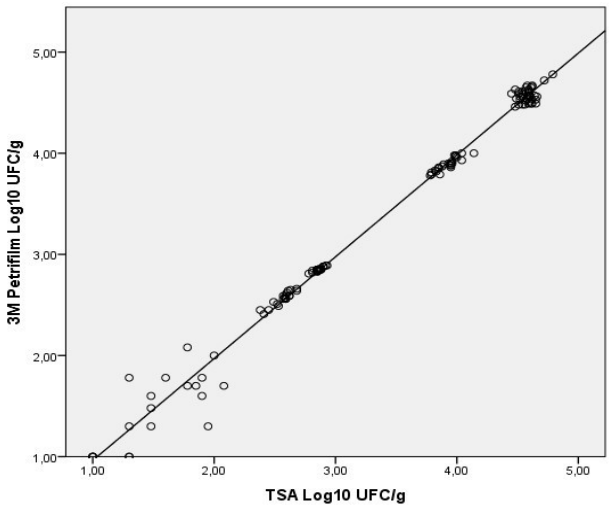

Figure 1. Linear regression obtained by comparing TSA and 3M-PetrifilmTM RAC.
Figura 1. Regresión lineal obtenida al comparar MSA y 3M-Petrifilm RAC.

In foods such as shrimp and pasteurized milk, according to Bird *et al.* (2016)(4) and in shrimp,

tomatoes, blackberries, sauce and dressings, fresh pasta, ice cream, powdered milk and pasteurized milk, Benzinger *et al.* (2014)(2), obtained similar results. A statistically significant correlation was found between the 3M-Petrifilm™ RAC method and the conventional TSA with a confidence level of 95%. Bird *et al*. (2016)(4) and Benzinger *et al*. (2014)(2) obtained high coefficients of determination, similar to those of the present study: R^2= 0.951 for raw shrimp, R^2= 0.997 for pasteurized milk, R^2= 0.9981 for ice cream, R^2= 0.9962 for sauces. No significant difference was found in microbial recovery with the cocoa product matrices observed between TSA and 3M-Petrifilm™ RAC. During the execution of the analyzes, no negative aspects were observed for the identification of the colonies. These were more easily identified on the 3MPetrifilm ™ RAC plates than in the conventional method due to the "colony color and intensity" observed during evaluation. 3M-PetrifilmTM RAC plates prevented spreading colonies (liquefaction), in the conventional method. This allowed easier enumeration on 3M-PetrifilmTM RAC plates than agar plates from the conventional method (4).

CONCLUSION

The 3M-PetrifilmTM RAC Alternative Microbiological Counting Method has been shown to be reproducible, reliable, and accurate.

Conflict of interest:

The authors declare that they have no conflict of interest.

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