Pesquisa Agropecuária Brasileira

Veterinary Science/ Scientific Notes

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

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Received
November 21, 2024

Accepted
March 31, 2025

How to cite
BOHRZ, D. de A.S.; LEVANDOWSKI, R.; SANTOS, L.F. dos; NASCIMENTO, C.A. do; PASQUALOTTO, L.; BERTUOL, M.Z.; NOVELLO, T.; PILOTTO, F.; SANTOS, L.R. dos; RODRIGUES, L.B. Efficacy of araçá extract in reducing biofilms on teat cup linings. **Pesquisa Agropecuária Brasileira**, v.60, e03972, 2025. DOI: https://doi.org/10.1590/S1678-3921.pab2025.v60.03972.

Efficacy of araçá extract in reducing biofilms on teat cup linings

Abstract – The objective of this work was to evaluate the efficacy of araçá (*Psidium cattleianum*) fruit extract in removing *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms from teat cup liners. New and used rubber liners were tested. Biofilm formation and removal were evaluated using a standardized protocol. The surface of the teat cup liners was analyzed with scanning electron microscopy. The used liners had a rougher surface, which promotes biofilm growth. The araçá extract outperformed sodium hypochlorite, being up to 21 times more effective in reducing biofilms on the used liners. Therefore, araçá extract shows potential as a biodegradable alternative for removing biofilms from teat cup liners.

Index terms: *Psidium cattleianum*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, dairy equipment hygiene, disinfection, natural extract.

Eficácia do extrato de araçá na redução de biofilmes em revestimentos de copos de teteira

Resumo – O objetivo deste trabalho foi avaliar a eficácia do extrato de fruta de araçá (*Psidium cattleianum*) na remoção de biofilmes de *Staphylococcus aureus* e *Staphylococcus epidermidis* em teteiras. Foram testadas teteiras de borrachas novas e usadas. A formação e a remoção dos biofilmes foram avaliadas com protocolo padronizado. Analizou-se a superfície das teteiras com microscópio eletrônico de varredura. As teteiras usadas apresentaram superfície mais rugosa, o que favorece o crescimento de biofilmes. O extrato de araçá superou o hipoclorito de sódio, com eficácia até 21 vezes maior na redução de biofilmes nas teteiras usadas. Portanto, o extrato de araçá apresenta potencial como alternativa biodegradável para remoção de biofilmes do revestimento de copos de teteiras.

Termos para indexação: *Psidium cattleianum*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, higiene de equipamentos lácteos, desinfecção, extrato natural.

Biofilms formed by *Staphylococcus aureus* and *Staphylococcus epidermidis* on dairy equipment, particularly on teat cup liners, are a significant concern for the dairy industry since they compromise milk quality and safety and increase the risk of mastitis in dairy cows (Feldmann et al., 2006). Although sodium hypochlorite, a common disinfectant, is often used to eradicate these biofilms, its potential toxicity and ineffectiveness against certain chlorine-resistant bacteria are among the faced challenges (Ózsvári & Ivanyos, 2022). Consequently, exploring alternative strategies to remove biofilms is

important to maintain bovine health and ensure milk quality.

Araçá (*Psidium cattleianum* Sabine), belonging to Myrtaceae family, whose fruit extract is known for its antioxidant and antibacterial properties, has been identified as a potential alternative agent for eradicating biofilms (Alvarenga et al., 2016). However, its effectiveness in removing staphylococcal biofilms, particularly in real scenarios, has not yet been studied.

The objective of this work was to evaluate the efficacy of araçá fruit extract in removing *S. aureus* and *S. epidermidis* biofilms from teat cup liners.

Two types of liners were analyzed: new and used; the latter in operation for approximately 2,500 hours. The biofilm substrates were prepared using commercially available teat cup liners made of nitrile butadiene rubber, cut into 1.0 cm^2 pieces and sterilized in an autoclave. For biofilm formation, 250 µL of the ATCC 25923 *S. aureus* or ATCC 12228 *S. epidermidis* bacterial suspension at 10^8 CFU per mL were mixed with 250 µL of sterile brain heart infusion broth in a 12-well plate containing a rubber coupon. The plates were incubated at 37°C and subjected to shaking for 24 hours.

To evaluate biofilm removal, the teat cup liners were washed with 5.0 mL of 0.1% peptone water to remove planktonic cells and then immersed in either sodium hypochlorite (12.5%) or araçá fruit extract for 10 min at room temperature; the used araçá extract was prepared from freeze-dried pomace using 80% ethanol in an ultrasonic bath at 30°C, as described by Santos et al. (2023). Afterwards, the treated teat cups were washed with 0.2% neutralizing peptone water and subjected to sonication for 10 min, at 40 kHz, in 5.0 mL of 0.1% peptone water to detach the biofilm. Detached cells were serially diluted, and bacterial concentrations were determined using the plate count method, expressed as log_{10} colony forming units per square centimeter. Surface morphology and roughness of the rubber teat cups were analyzed using scanning electron microscopy (SEM), with images acquired at various magnifications to compare the new and used liners.

Staphylococcus epidermidis produced 15.40% more biofilm than *S. aureus* on new rubber and 11.40% more on used rubber (Figure 1). This could be attributed to the more prevalent colonization of the skin by *S. epidermidis*, as well as to the bacteria's capacity to

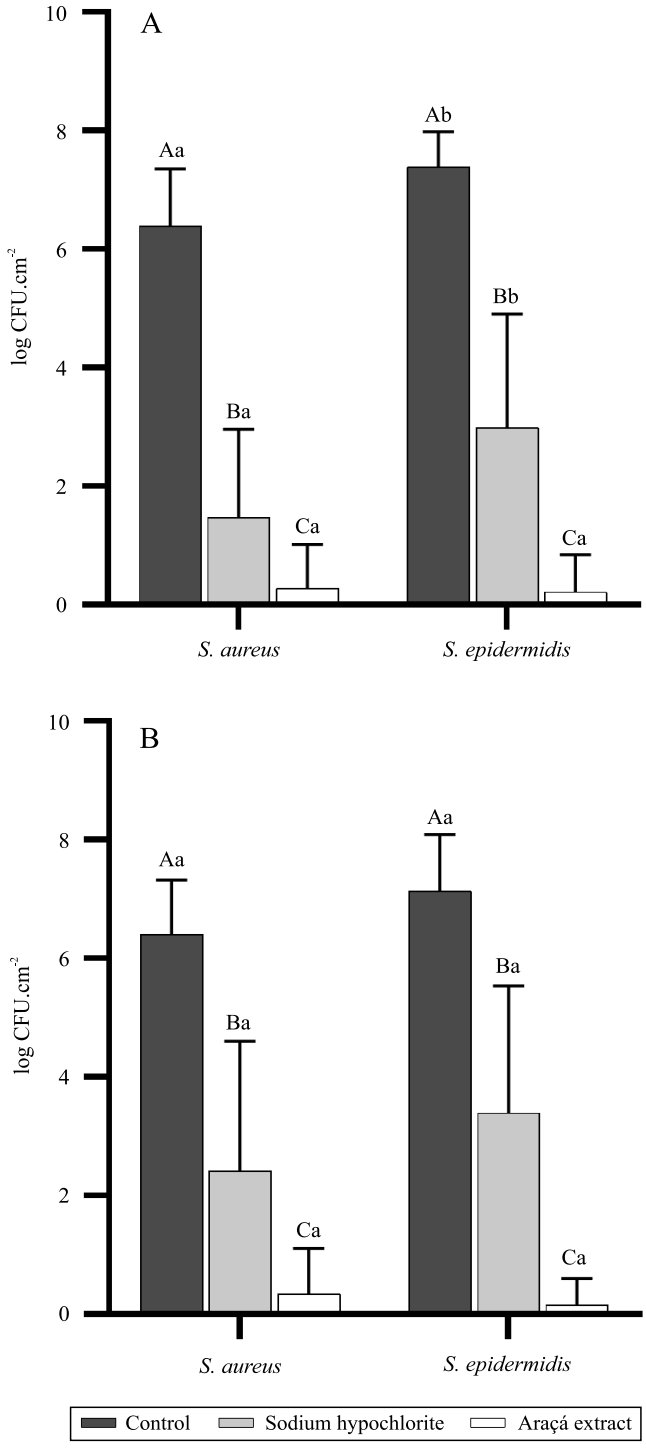

Figure 1. Effect of sodium hypochlorite and araçá (*Psidium cattleianum*) fruit extract on biofilms of *Staphylococcus aureus* and *Staphylococcus epidermidis* on new (A) and used (B) rubber of teat cup liners. Different uppercase letters indicate significant differences ($p<0.05$) among treatments within the same bacterial species, and different lowercase letters indicate significant differences ($p<0.05$) between bacterial species within the same treatment.

generate polysaccharide intercellular adhesin and polymeric N-acetyl-glucosamine, which facilitate biofilm formation and resistance (Rohde et al., 2010).

In terms of biofilm removal, the extract from the araçá fruit outperformed sodium hypochlorite in reducing the bacterial counts of both *S. aureus* and *S. epidermidis* on rubber (Figure 1). Specifically, the araçá fruit extract was 5 times more effective at removing *S. aureus* biofilms and 14 times more effective at removing *S. epidermidis* biofilms from new rubber. On used rubber, the extract was 7 times more effective at removing *S. aureus* biofilms and 21 times more effective at removing *S. epidermidis* biofilms.

Regardless of the bacterial strain, biofilm formation was more prevalent on used rubber cups than on the new ones due to differences in their surfaces (Figure 2). The SEM images showed that the new rubber had a smooth and uniform surface, while the used rubber had a rough and heterogeneous surface with many cracks, pores, and irregularities. This deterioration of the rubber surface over time, due to factors such as mechanical stress, chemical agents, and environmental conditions, can reduce the mechanical strength and elasticity of the rubber, while increasing its permeability and roughness, which promotes bacterial attachment and biofilm growth (Gálik et al., 2015). Therefore, modifications in the structure of the teat cup liners can significantly impact their performance and cleanliness, potentially leading to the formation of biofilms.

Through the results obtained in the present study, the araçá fruit extract, given its antimicrobial properties and biodegradability, showed effectiveness in reducing the bacterial populations on the teat cup liners, surpassing the traditional disinfectant sodium

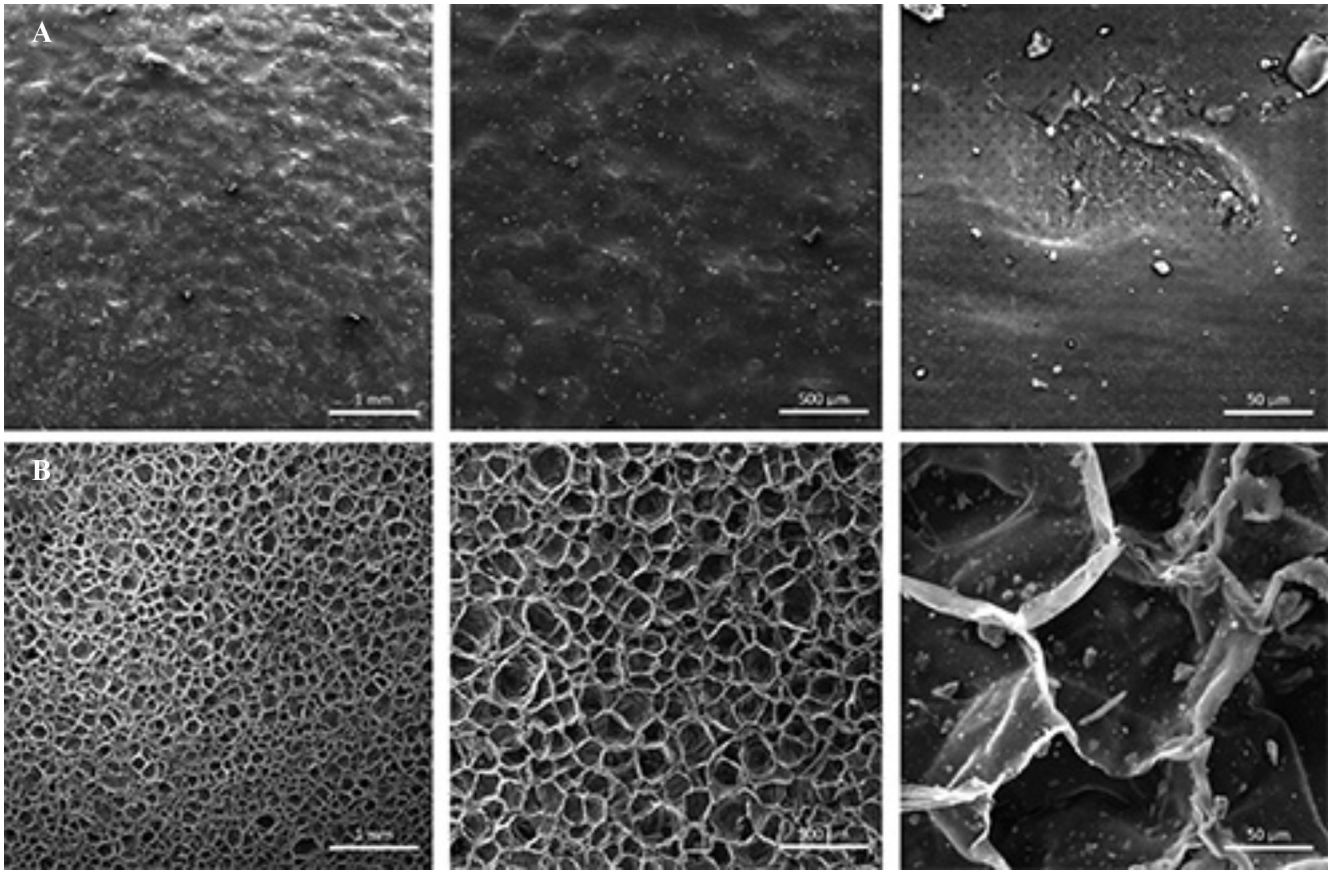

Figure 2. Scanning electron microscopy images of new (A) and used (B) rubber of teat cup liners at magnifications of 50, 100, and 1000x, showing a relatively smooth and uniform surface and a rougher and more heterogeneous surface, respectively.

hypochlorite by a factor of up to 21. Therefore, the araçá extract could replace synthetic chemicals as a natural solution for biofilm management in dairy farming.

Acknowledgments

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for financing, in part, this study (Finance Code 001).

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