

TITLE: INFLUENCE OF CULTURE MEDIA ON THE INACTIVATION OF *BACILLUS* SPP. IN PROCESSED CHEESE: IN VITRO EVALUATION

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ABSTRACT:

Polyphosphates are emulsifying agents used in the manufacture of processed cheeses. These salts can also act as preservatives, but its inhibition may be compromised when it's added to culture media. In this study, we verified the interference of four culture media in microbial inhibition by the emulsifying salt JOHA® HBS (ICL, Ladenburg, GER). The enumeration assay was performed on Brain Heart Infusion Agar (BHI, Oxoid, Basingstoke, UK), Nutrient Agar (NT, Oxoid), Plate Count Agar (PCA, Oxoid) and Trypticase Soy Agar (TSA, Oxoid) adding different concentrations of JOHA® HBS salt (A1: 0.5%; A2: 1%; A3: 2% HBS; AC: control without HBS). Each solid media was prepared according to the manufacturer's instructions. After homogenization we simulated the manufacturing of processed cheese by heating the culture media (85 °C/15 min) and then poured into Petri dishes. The inoculum of three strains (*Enterococci faecalis* FAIR-E 179, *Listeria monocytogenes* Scott A and *Staphylococcus aureus* ATCC 6538) was standardized to a concentration end of approximately 3×10^6 cfu/mL of each stain was inoculated in the Petri dishes and incubated at 37 °C for 24 h. Viable cell counts were determined at 0 h and after 24 h of incubation. The typical colonies were enumerated and the results expressed as colony-forming units per mL. Counts were converted as \log_{10} and results compared considering treatments by ANOVA and Tukey honestly significant difference (HSD) test ($P < 0.05$). For *E. faecalis*, no significant differences were observed between the concentrations tested in BHI and TSB media, noting that polyphosphate did not affect the growth of *E. faecalis* in these media and lower cell density was observed in the concentrations of 1% and 2% (w/v) in NT and PCA. For *L. monocytogenes*, the data shows that salt inhibited bacterial growth in any media tested with 2% (w/v) of the salt. The NT media was the one with the lowest cell density for all concentrations of the salt that was tested. For *S. aureus* similar results were obtained, however at the concentration of 2% the viable counts were only significantly different in BHI, in which the count was the highest. The reduction in the effect of polyphosphates can occur due to the presence of multivalent metal ions in the culture medium, the number of phosphorus atoms and acid-base properties of phosphates in aqueous solutions. Therefore, when the JOHA® HBS salt is used, the NT media appears to be the one with minor interferences in cell growth.

Keywords: polyphosphates; cell inhibition; preservatives;

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