

TITLE: PHOTOTOXIC POTENTIAL OF CURCUMIN IN A *IN VITRO* PHOTODYNAMIC INACTIVATION OF *PSEUDOMONAS FLUORESCENS*

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ABSTRACT:

Pseudomonas fluorescens (*P. fluorescens*) is the main deteriorating psychrotrophic species of milk and dairy products, capable of proliferating at low temperatures, form biofilms and produce thermostable enzymes that negatively affect sensory quality. Photodynamic inactivation of microorganisms (PDIM) is a technique for the formation of reactive oxygen species (ROS) through photosensitizing compounds (PS) activated by a visible light photon. This technology is still little explored in the food industry and, therefore, the objective was to determine *in vitro* the phototoxic potential of Curcumin (CUR; 1, 7-bis-(4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione) against *P. fluorescens*. For this, a solution of CUR at the concentration of 125 µg/mL was prepared under stirring using 2.5% of dimethylsulfoxide. The inoculum of *P. fluorescens* was standardized at 0.5 on the McFarland scale, corresponding to 8 logCFU/mL. In a 96-well plate, 90 µL of the CUR solution was added to the first well, followed by decimal dilution in subsequent wells. Each well was inoculated with 10 µL of standardized bacterial suspension. The samples remained in the dark for 10 min and then were subjected to illumination for 5 min in a blue light emitting diode ($\lambda_{\max} = 450$ nm, optical power of 2.7 mW cm⁻²) with a total energy dose of 0.81 J cm⁻². Subsequently, serial dilutions of the samples were inoculated in Pseudomonas Agar Base and incubated at 25 °C/48 h. To control the light and the solubilizing medium, the suspension of microorganisms was irradiated in the absence of CUR and inoculated in a solution without CUR. Application of light without CUR had no effect on microbial growth. The concentration of CUR interfered with the efficiency of inoculum inactivation, with greater reductions for higher concentrations. CUR showed toxicity in the dark, but not enough to inactivate 100% of the inoculum. The combination of 62.5 µg/mL of CUR and illumination inactivated 100% of the inoculum (*P. fluorescens* 7 logCFU/mL). This is because the excited PS transfers energy to molecular oxygen (O₂), forming highly toxic ROS, capable of reacting with biomolecules and cell structures, resulting in cell damage, such as membrane rupture and cytoplasm leakage. Therefore, CUR has dark toxicity and synergistic activity against *P. fluorescens* when combined with lighting, and PDIM can be considered an alternative technique for use in milk and dairy products.

Keywords: dairy products, food contamination, food preservation, milk, psychrotrophic bacteria