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RELEASE OF POLYPHENOLS FROM CARNAUBA WAX NANOPARTICLES

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The inclusion in foods of compounds with antioxidant activity from rich sources (fruits, aromatic herbs, etc.) has become a common procedure of the food industry. However, when incorporated in food matrix these compounds may interact with matrix components and reduce or loss bioactivity. Hence, the formulation of loaded polyphenols nanoparticles may offer a way to protect such compounds against degradation. In this work, the releasing rates from Solid Lipid Nanoparticles (SLNs) of rosmarinic acid (RA) were tested. Moreover, the effect of selected SLNs upon DNA *i.e.* antioxidant and pro-oxidant effects were also evaluated. Different formulations of SLNs were tested, using 0.5% (w/v) of carnauba wax and 1 and 2% (v/v) of polysorbate 80, prepared by a hot melt ultrasonication method.

Two types of *in vitro* release tests were performed. The first approach used a cellulose acetate dialysis bag with molecular weight cut-off of 12 kDa, to evaluate the direct release of RA from the SLNs. The experiment was conducted during 12 h, at 37 ± 0.5 °C with continuous homogenization. The SLNs were dispersed in PBS (0.1 M, pH 7.4), and samples were withdrawn at different time points during the experiment (0, 1, 2, 4, 6, 8, 10 and 12 h). The polyphenol content of samples was analysed by HPLC. The second test using intestinal-based cell co-culture (Caco-2 and HT29-MTX cells), was performed to study the cells permeability to the RA and the possible presence of secondary metabolites from RA. The passage through the cell monolayer and cytotoxicity were evaluated by HPLC and TEER monitoring, respectively. The SLNs were diluted in sterilized ultrapure water, the solution was placed in the apical zone of the cells and samples were taken from the sub-apical zone, at different times.

In both *in vitro* release tests the results have shown high % of RA release. The test using dialysis bag showed 50% RA release after 4h of experiment, and by 12h the % of release reached ca. 90%. On the other side, tests using intestinal cell lines showed that the Caco-2 cells have higher % of passage of RA (ca. 60%) for the sub-apical zone, when compared with the set of Caco-2 and HT29-MTX cells.

Finally, SLNs do not attack DNA chains, but also do not protect the DNA, demonstrating that SLNs have a neutral effect, whereas free RA demonstrated a protective effect on the DNA chain.