

# Phytoestrogen coumestrol: Antioxidant capacity and its loading in albumin nanoparticles

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Coumestrol is a polyphenol with promising therapeutic applications as phytoestrogen, antioxidant and potential cancer chemoprevention agent. The presence of two hydroxyl groups on its chemical structure, with orientation analogous to estradiol, is responsible of both, its antioxidant capacity and its estrogenic activity. However, several studies show that the interaction of polyphenols with food and plasma proteins reduces their antioxidant efficacy. We studied the interaction of coumestrol with bovine serum albumin protein (BSA) by fluorescence spectroscopy and circular dichroism techniques, and the effect of this interaction on its antioxidant activity as a hydroxyl radical scavenger. In addition, coumestrol antioxidant capacity profile using different assays (DPPH, ORAC-FL and ORAC-EPR) was studied. To explain its reactivity we used several methodologies, including DFT calculations, to define its antioxidant mechanism. Coumestrol antioxidant activity unveiled interesting antioxidant properties. BSA interaction with coumestrol reduces significantly photolytic degradation in several media thus preserving its antioxidant properties. Results suggest no significant changes in BSA structure and activity when interacting with coumestrol. Furthermore, this interaction is stronger than for other phytoestrogens such as daidzein and genistein. Considering our promising results, we reported for the first time the fabrication and characterization of coumestrol-loaded albumin nanoparticles. The resulting spherical and homogeneous nanoparticles showed a diameter close to 96 nm. The coumestrol incorporation efficiency in BSA

NPs was 22.4%, which is equivalent to 3 molecules of coumestrol for every 10 molecules of BSA.