

Title: Liposome encapsulation of a oleuropein-rich olive leaf extract and study of the effect of oleuropein on model lipid membrane

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The large increase in number of food products enriched with bioactive compounds having health promoting properties while relatively unstable, has been promoted by the development of encapsulation technologies allowing protection and targeted delivery. Olive leaves are a waste product of olive oil processing with high concentration of phenolic compounds, being oleuropein the most representative, with remarkable health promoting properties.

In this study, OLE was encapsulated into liposomes by a simple and food grade method. Liposomes were characterized using TEM and z-size/potential. Stability and release of oleuropein from liposomes was studied at 5°C and 90°C in model systems at different pH values and time, and in a model/commercial lemonade drink kept at different temperature. Furthermore, the effect of oleuropein on the fluidity of a model lipid liposome membrane at different oleuropein/lipid molar ratios was evaluated by means of differential scanning calorimetry and fluorescence anisotropy.

OLE-liposomes presented a diameter ranging from 100 to 600 nm as observed with electron TEM and, with an average diameter of  $485 \pm 9$  nm. Encapsulation efficiency of oleuropein, main OLE compound, was  $33.8 \pm 1.5\%$ , while that of verbascoside encapsulation efficiency was  $75.0 \pm 0.8\%$ . An early release of 15-20% oleuropein during the first hour followed by a slow sustained release was observed at 5°C.

Liposome encapsulation slowed down oleuropein degradation in acidic pH 2, while slightly enhanced oleuropein stability in the model lemonade drink at 90°C; however, it did not have an effect on OLE stability at 5°C and in the commercial lemonade drink. The main phase transition of model lipid membranes showed a splitting and gradual shift to lower temperatures and a decrease on the pre-melting transition enthalpy when oleuropein was present at a 1:1 oleuropein/lipid molar ratio or higher, indicating that oleuropein is likely to be partially inserted into lipid bilayer.