Interfacial Activity and Encapsulation Characteristics of Oleuropein and Olive Leaves Extracts in Micro/nanodispersions

（マイクロ・ナノ分散系におけるオレウロペインとオリーブ葉抽出物の界面活性とカプセル化特性）

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ABSTRACT

Olive leaves are an abundant and low-cost natural raw material and are rich in phenolic compounds, mainly oleuropein, claimed for countless health benefits. Ongoing problems on the use of olive leaves for food fortification are the bitter taste of oleuropein and its low bioavailability in the intestinal gut. Encapsulation of oleuropein in micro/nanodispersions could be an attractive strategy to overcome these problems. Emulsions, which are a type of micro/nanodispersion system, are potential candidate for encapsulation purposes because of their wide presence as structure forming entities in the design of advanced foods and drugs. Emulsions are composed of two or more immiscible liquids and require the presence of an emulsifier layer that stabilizes droplets. To develop the emulsions suitable for oleuropein delivery on a more quantitative and qualitative basis, it is outstandingly important to take into account any possible interactions that may occur between oleuropein and the various emulsion components, mainly at the interface. On this basis, it is imperative to study the oil-water interfacial properties of oleuropein in order to design the suitable emulsion system for oleuropein encapsulation. This work intends to investigate the interfacial properties and the encapsulation characteristics of olive leaves extracts (OLEs) and oleuropein in micro/nanodispersions systems.

The interfacial properties of OLE and highly purified oleuropein were investigated by interfacial tension measurements. The results demonstrated that oleuropein is a highly interfacial-active compound in the OLE because of its pronounced ability to decrease the interfacial tension at the triglyceride oil-water interface. The packing of oleuropein molecules at this interface was estimated on the basis of their surface excess concentration and area occupied per molecule, determined from the Gibbs adsorption theory.

Based on the preceding results, the emulsifying ability of oleuropein to stabilize triglyceride oil-water (O/W) emulsions was investigated using microchannel (MC) emulsification. The results demonstrate the successful preparation of monodisperse, oleuropein-stabilized O/W emulsions with a coefficient of variation (CV) of <5% when using triglyceride oils of higher hydrophobicity, even in the absence of the cross-flowing continuous phase. MC emulsification using oleuropein as the emulsifier was also capable of continuously preparing monodisperse O/W emulsions for 15 h, if appropriate triglyceride oils are used.

The dynamic behavior of oleuropein in vacuum, water and oil-water systems was studied through molecular dynamics (MD) simulations at 300 K and for at least 30 ns. The seven dihedral angles that describe the flexible skeleton of oleuropein were monitored together with the distance between glucose and hydroxytyrosol. The obtained trajectories demonstrated that oleuropein adopts different conformations that depend on the environment. In triolein/water system, oleuropein adopts an opened form where the glucose moiety could be aligned with hydroxytyrosol and elenolic acid moieties. Water mediated hydrogen bonding were greatly

involved in stabilizing the three dimensional conformation of oleuropein. The absence of hydrogen bonding with water molecule in the elenolic acid moiety of oleuropein might be the key towards its amphiphilic character and its stabilization at the triolein-water interface.

Oleuropein is known for its antimicrobial activity against a wide spectrum of bacteria, fungi and virus. Despite the high interest of its use in food, little is known about the possibility of a direct relationship between the antimicrobial activity and the interfacial activity of oleuropein in O/W emulsions. Therefore, the antimicrobial activity of O/W containing oleuropein and different food grade emulsifiers (Tween 80 and decaglycerol monolaurate (ML750)) was assessed against the Gram negative *Escherichia coli* JCM 1649 microorganism. The results demonstrated that the antimicrobial activity of a surface active polyphenol such as oleuropein is dependent on the composition of the O/W emulsions, mainly on its total interfacial area, that is proportional to the emulsifier concentration in the O/W emulsions. The lower activity of oleuropein in O/W emulsion was experimentally proved due to its sequestering at emulsion interface that could be a sink or a source of the antimicrobial activity.

The encapsulation characteristics of oleuropein in W/O/W emulsions were also investigated using two-step emulsification technique consisting of high-pressure homogenization and subsequent MC emulsification. Monodisperse oleuropein-loaded W/O/W emulsions with average W/O droplet diameters of around 27 µm and CV below 5% were successfully prepared by MC emulsification. Due to the interfacial activity of oleuropein, it is needed to increase the concentration of the internal hydrophobic emulsifier to promote higher encapsulation efficiency and higher stability of oleuropein loaded-W/O/W emulsions.

The findings obtained from this study are believed to provide new insights for novel food and pharmaceutical applications of oleuropein as a natural emulsifier. Furthermore, these findings also suggested that the surface activity of a bioactive is a key parameter in optimizing the formulation and the biological activities of emulsion systems for food and drug applications.