ABSTRACT

The increasing consumer concern and demand in the last decades for foods with natural ingredients and enhanced nutritional and health-promoting properties has pushed the research in the fields of nutrition and food technology in order to find, characterize, formulate and incorporate bioactive compounds into common foods or nutraceuticals. Some of the most used food bioactives are plant phytochemicals like polyphenols present in most plants as secondary metabolites. Olive biophenols, present in most parts of the plant and found in high concentrations in the leaves, are widely recognized for their bioactive properties. Thus, olive leaves, traditionally considered as a by-product, are an abundant source of olive phenolic compounds that can be recovered and further exploited as functional ingredients in food and nutraceuticals.

However, the intrinsic chemical characteristics of these and other phytochemicals compounds makes its direct use in foods very challenging. In this context, food microencapsulation has been progressively implemented to partially or totally tackle these technological and sensorial issues associated to their incorporation into foods. The large variety of bioactives (pure compounds, phenolic-rich extracts) and carriers, the specific objective of the encapsulation and the large complexity of food matrices are the reasons for the wide research and development approaches in this field. Due to the different nature of the encapsulation techniques, specific investigations on their related critical parameters and optimization of the encapsulation process and performance are needed.

The investigations carried out in this work aimed to better understand the use of phenolic extracts from olive leaves by studying its chemical stability and developing

encapsulated ingredients with encapsulation techniques of different nature with the potential to be used in real food matrices.

Firstly, a phenolic extract from olive leaves rich in oleuropein, a well defined compound with significant health-promoting properties, was characterised in terms of thermal stability at varying pH conditions of the phenolic content, profile and radical scavenging properties, by implementing a kinetic model and also structural characterization using flurescence spectroscopy.The thermal degradation of the major component, oleuropein, followed first-order kinetics and was high at lowest pH values (pH = 2), while verbascoside appeared to be more labile at pH 6. Oleuropein hydrolysis products resulted in an increasing hydroxytyrosol concentration, that followed zero-order kinetics. These changes were also detected by fluorescence spectroscopy. On the other hand, no remarkable changes in total phenolic content and radical scavenging activity were observed.

Freeze-drying was studied as a method to encapsulate olive leaf bioactives in amorphous dry carbohydrate matrices .The effect of the carrier formulation and ratio bioactive:carrier on the encapsulation efficiency, the thermal, physical and structural properties of freeze-dried microencapsulated powders was assessed by using a response surface modellig approach. Also, the impact of these factors on the chemical stability of bioactive compounds was studied. Maltodextrin and trehalose were chosen as encapsulating materials as representatives of high and low molecular weight carbohydrates with good glass forming properties for encapsulation purposes. The increasing concentration of maltodextrin enhanced the encapsulation of both total phenolics and oleuropein up to an almost total retention when maltodextrin was used alone, which could be directly observed thanks to fluorescence imaging. Color and thermal properties of the microencapsulated powders depended on the maltodextrin-trehalose ratio and a plasticizing effect of olive leaf extract was also observed, especially in the

glassy powders containing maltodextrin. The storage study of unencapsulated and microencapsulated olive leaf extract powders under different physical states revealed that at least for 7 weeks, the chemical stability and antioxidant properties of the bioactives were not affected.

Liposomal encapsulation of olive bioactives was investigated, first in model phospholipid membranes by evaluating the effects of oleuropein on membrane thermotropic behavior (differential scanning calorimetry) and ordering and fluidity (fluorescence polarization) in systems with passively encapsulated oleuropein (i.e., added after formation of liposomes) compared to actively encapsulated oleuropein (i.e., encapsulated during formation of liposomes). Also, the antioxidant capacity of oleuropein to inhibit lipid peroxidation. was evaluated under two types of oxidation induction. A potential food ingredient was developed by encapsulating the olive leaf extract in commercial soy phosphatidylcholine, and characterized for its morphological, physical and functional properties in model and real systems (commercial soft drink). Oleuropein and verbascoside were encapsulated with a mean efficiency of 34% and 75%, which indicated that optimization this process can be further investigated to improve the encapsulation. However, liposome encapsulation was effective for a delay of oleuropein degradation at low pH (i.e., pH 2.0), and for the maintenance of oleuropein stability over long periods at refrigeration temperatures and at different pHs. This thus shows that this lipid encapsulation indeed provides a suitable carrier in food systems, such as beverages.

Finally, the antioxidant performance of olive leaf extract and other standard and plant extracts was assessed in more challenging and real food processing conditions like those commonly employed during melt-extrusion processing/encapsulation, as opposed to classical liquid antioxidant assays. A simple and novel approach has been proposed to estimate the antioxidant

performance under controlled conditions using a lab-scale extruder by implementing a solid-state adaption of the crocin-bleaching liquid assay, based on the bleaching of saffron crocins.