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Development of Encapsulated Olive Oil and Olive Extracts as Innovative Natural and Health Food Ingredients

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This PhD project is aimed to develop ingredients with encapsulated olive bioactives (olive leaf extract) rich in phenolic compounds with health properties, with special attention to the main compound oleuropein. Several encapsulation techniques have been applied, namely freeze-drying, co-milling and liposomes, to produce products with different properties. Encapsulation efficiency, quality, stability and technological functionality were evaluated in both simple and complex model systems.

Sviluppo di incapsulati a base di olio ed estratti di oliva per la produzione di ingredienti funzionali e salutistici

Questa tesi di dottorato ha riguardato lo sviluppo di ingredienti incapsulati di composti bioattivi di olivo (estratti di foglie) ricchi di composti fenolici con proprietà salutistiche, con particolar attenzione al composto principale, oleuropeina. Diverse metodiche di incapsulamento sono state usate: liofilizzazione, milling e liposomi, con caratteristiche diverse. L'efficienza d´incapsulamento, la qualità e la stabilità e le proprietà tecnologiche e funzionali, sono state valutate sia in sistemi modello semplici che in sistemi complessi.

Key words: microencapsulation, olive leaves, oleuropein, freeze-drying, amorphous carbohydrates, liposomes

1. Introduction

In accordance with the PhD thesis project previously described (González Ortega, 2017), this oral communication reports the main results of the following four activities directed to:

A1) **Development of encapsulated polyphenolic olive extracts using different techniques**

A1.1. Freeze-drying encapsulation (University of Teramo)

A1.2. Liposome encapsulation (University of Ljubljana)

A2) **Evaluation of technological functionality and stability of produced encapsulated extracts,** including encapsulation efficiency, microstructure and morphology (microscopy), thermal stability (calorimetry), water sorption, bioactive release and stability.

A3) **Determination of stability, health functionality and bioavailability in real food systems:** development of model and formulated emulsified and gelled products

2. Microencapsulation

Throughout last decades significant changes occurred in food production, now not only oriented to meet basic nutritional needs and sensory properties but increasingly focused on prevention of nutrition related diseases and well-being of consumers (Roberfroid, 2002). Main attention is given to presence and concentration of minor compounds able to exert bioactivity of various nature and health-promoting properties in humans, whose interest is increasing also for the likely exploitability as therapeutic agents and nutraceuticals. This interest has powered the research and development of new technologies to keep and enhance bioactive compounds in food products, to improve their bioavailability as well as to protect them against stressing environmental and matrix factors (McClements, Decker, Park, & Weiss, 2009) . Micro-encapsulation or nano-encapsulation is nowadays representing an interesting strategy to enhance the functionality of bioactives and other biomolecules. From a food technological perspective, it is essential to maintain physicochemical quality and bioactivity of such compounds during prolonged storage and various storage conditions. It is also more convenient and applicable to benefit from bioactive phenolic extracts in powdered form that can be easily handled during storage and transportation and applied to in food and pharmaceutical purposes (Fang & Bhandari, 2012)

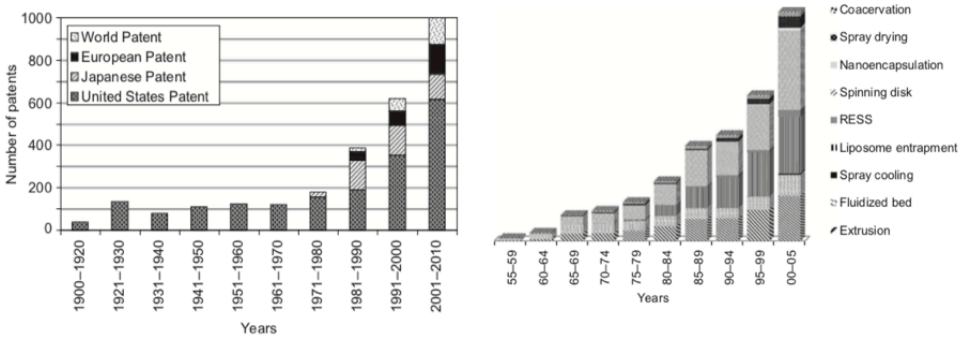

Figure 1. *Trends in microencapsulation and number of patents during last decades. Note: Sourced from Sobel, R., Versic, R., & Gaonkar, A. G. (2014). Introduction to microencapsulation and controlled delivery in foods. In Microencapsulation in the Food Industry (pp. 3-12).*

2.1. Freeze-drying encapsulation and effect of processing variables

Freeze-drying is a widely applied technique to encapsulate phenolic compounds and other heat-sensitive compounds, by means of co-lyophilization of a solution containing the bioactive of interest and a carrier/wall material, resulting in a dry, porous structure (Fang & Bhandari, 2012).

The application of freeze-drying requires a preliminary optimisation of the process factors such as wall material selection, amount of bioactives-wall, drying conditions to understand and tune the qualitative and technological properties of the microencapsulated powders. Commonly used wall materials are polysaccharides such as chitosan and alginate, and other carbohydrates like starch, maltodextrins and smaller oligosaccharides, thanks to their ability to form amorphous glassy matrices held up by hydrogen bonds (Kilburn, Claude, Schweizer, Alam, & Ubbink, 2005) in which the core bioactive is entrapped. Molecular weight and type of carbohydrate as well as presence of a second or third component in the amorphous solid matrix are known to affect the microstructure and

physical properties (i.e. glass transition temperature), that governs molecular mobility and diffusion-controlled reactions (Roos & Drusch, 2015). Several studies have addressed encapsulation of vegetable and fruit extracts with maltodextrins with varying molecular weight (i.e dextrose equivalent). However, limited is the information about the impact of freeze-drying process variables on encapsulation efficiency, powder microstructure, micro-distribution of bioactives and glass transition of resulting microencapsulated powders
Response surface methodology is a useful design tool employed to optimize and study processes that have several factors and factor interactions influencing the responses, and has been commonly applied in encapsulation (Paulo & Santos, 2017).

2.2. Liposome encapsulation

Liposome particles are highly functional delivery systems for drugs and bioactive compounds. They are vesicles formed by lipid bilayers usually by using phospholipids and have remarkable advantages mainly due to their biocompatibility and possibility to tune their physicochemical properties. Encapsulation of food bioactive compounds with liposomes proved to improve stability, slower release and higher radical scavenging capacity in vitro, compared to free form of phenolic compound. Despite these effects and the possibility to prepare them using only natural food grade components, their application in food systems is still limited due to time consuming manufacturing processes and high costs. Recent studies are evaluating the encapsulation of food bioactive compounds using the proliposomes method (Perrett, Golding, & Williams, 1991) in which undesired solvents and expensive procedures are avoided, allowing scalability and food grade applications.

The behaviour and interaction of phenolic compounds with model lipid membranes and its effect on their physical and chemical state can provide valuable information to understand the techno-functional properties of encapsulated bioactive. Moreover, liposomes can serve as valuable models to understand protective effect of phenolic compounds against oxidative damage of lipid membranes (Balanč et al., 2015).

4. Experimental Procedure

In this PhD thesis dissertation, two encapsulation techniques were applied to encapsulate an olive leaf phenolic extract (OLE). Firstly, freeze-drying was applied and the effect of formulation variables on encapsulation efficiency, microencapsulates microstructure and distribution of bioactives in the amorphous matrix and glass transition. A response surface methodology was applied using a central composite to optimize the design of experiments and information obtained.

Secondly, liposome encapsulation was used, and two studies were carried out. A study on the effect of oleuropein (as main OLE bioactive compound) on model lipid membrane physicochemical properties was carried out to gain insight on the role of oleuropein in membrane stability and fluidity, its impact on techno-functionality, and its role against lipid peroxidation. A parallel study dealt with a further applicative approach in which OLE-liposomes were prepared and characterized with regard to encapsulation of main phenolics, size and charge and morphology. Oleuropein stability and release from liposomes was studied at different pH and temperature condition and in real/model lemonade drink.

5. Materials and Methods

A standardized olive leaf extract with a high content of oleuropein (~40 wt.%) kindly provided by OLEAFIT S.r.l (Italy), was used as bioactive core material.

Table 1. *Overview of expected bioactive components and wall carrier materials.*

Encapsulation method	Bioactive core	Wall/carrier material
Freeze-drying	OLE powder	Maltodextrin, Trehalose and their mix
Liposomes	OLE powder Oleuropein	Soy lecithin (Phospholipon 90G) Dipalmytoylphosphocholine (DPPC)

Freeze-drying encapsulation: A design of experiment (DoE) and a central composite design with inscribed star points and response surface methodology was applied to produce OLE encapsulates. Three formulation parameters were taken as independent variables to produce encapsulates, namely: (V1) % total solids, (V2) composition ratio of matrix components and (V3) core:wall ratio. Microencapsulated dried powders were tested for: encapsulation efficiency, antioxidant capacity, colour, glass transition temperature (T_g) (DSC), surface morphology (SEM) and microdistribution of OLE (CLSM).

Liposome encapsulation: Pure oleuropein was used to study polyphenol-lipid membrane interaction and the effect on physicochemical properties and stability of the corresponding model membranes (liposomes). Thermal phase transitions were evaluated with a N-Differential scanning calorimetry (N-DSC); membrane fluidity and ordering by using fluorescence polarisation. Membrane lipids peroxidation was determined with BODIPY 581/591 C11 fluorescent probe and TBARS assay.

6. Results and Discussion

6.1 Freeze-drying encapsulation

The statistical analysis of the results of the TPC and oleuropein content indicated that the encapsulation efficiency (EE) of OLE was most significantly affected by matrix composition and ratio core: wall. Higher EE were obtained as the percentage of maltodextrin as carrier material increased, indicating that maltodextrin exert a better entrapping capsule material compared to trehalose. Additional factors that could be implied in this result include also potential interaction between the OLE phenolic compounds and the larger carbohydrate molecules of the maltodextrins and trehalose, affected by the specific charge, solubility and molecular mobility. It has been suggested that the plasticizing role of small sugars in amorphous polysaccharides is related to a reduction in the number of molecular entanglements without significantly changing the structure or packing of the glass (Kilburn et al., 2005). The ratio of bioactive core to matrix carrier (core: wall, X_3) also was a significant factor. The lower the ratio, i.e., the higher the matrix fraction in relation to the bioactive core material, the higher the encapsulation efficiency.

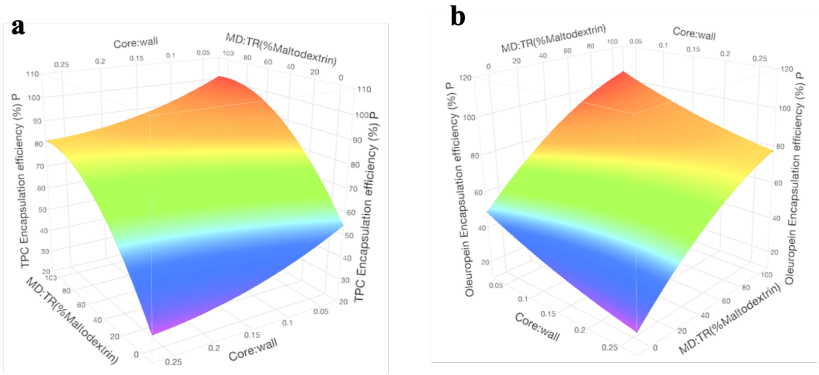

Figure 2. *Response surface 3D plots for encapsulation efficiency of (a) total phenolic content (TPC) and (b) oleuropein as a function of changes in factors V2 (ratio MD:TR) and V3 (ratio core: wall).*

Matrix composition affected also the microdistribution of OLE in the powders as shown by the CSLM images (Figure 3). In particular, a higher fluorescence signal was observed in sample with higher trehalose content. This may be due to higher surface content of phenolics in these sample, as observed in encapsulation efficiency, since highly entrapped phenolics may have impaired or quenched fluorescence emission as compared to surface fraction.

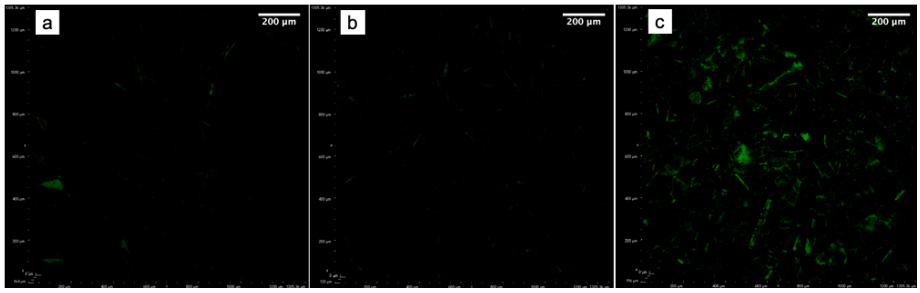

Figure 3. *CLSM micrographs of microencapsulated powders at OLE:Matrix ratio 0.05 and (a) 100% maltodextrin, (b) 50%-50% maltodextrin-trehalose and (c) 100% trehalose as wall material.*

The glass transition temperature of a system and its variation as a consequence of either matrix modification or enrichment with bioactive components represents one of the most important landmark for predicting the physical and chemical stability of low-moisture foods (Roos & Drusch, 2015). A single glass transition was observed in binary matrix samples (MD+TR), confirming its miscibility. OLE affected the Tg of the powders in which it has been physically entrapped by decreasing that of the correspondind carrier system without the extract. This effect may be a result of a plasticizing effect of the OLE low molecular weight components

6.2 Liposome encapsulation

DSC measurements were used to evaluate the changes in thermotropic properties of the liposomes membranes in the presence on encapsulated or titrated oleuropein. DPPC was used as a pure and saturated phospholipid with a sharp phase transition that can be easily monitored Figure 4. The changes in enthalpy (ΔH), gel-to-liquid phase transition (T_m)

and its related pre-transition (T_{pre}) were measured as a function of oleuropein:lipid molar ratios. It was observed an oleuropein concentration-dependent decrease in ΔH of T_{pre} and a broadening and shifting to lower temperatures of T_m without a significant change in ΔH. When oleuropein was encapsulated, this trend was amplified, showing an eventual disappearance of T_{pre} and a notable decrease of T_m from 41.6ºC to 40ºC.

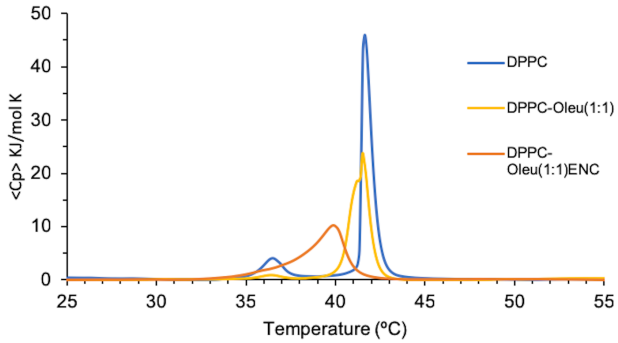

Figure 4. *DSC thermograms of DPPC liposomes in the absence and presence of oleuropein at 1:1 molar ratio, added during liposome formation (ENC) or after liposome formation.*

Results highlight that when oleuropein was encapsulated, it was partially intercalated in the phospholipid membrane and has stronger impact on membrane fluidity acting as a spacer.

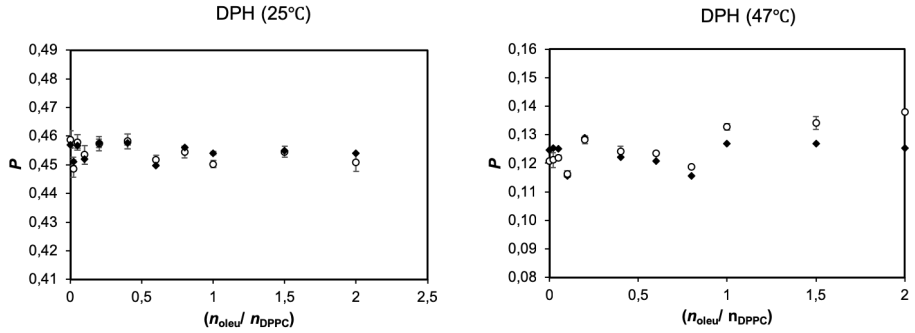

Figure 5. Fluorescence polarisation of DPH in liposome suspensions and titrated with increasing concentrations of oleuropein, below (25ºC) and above (47ºC) the T_m.

Fluorescent labels added to liposomes with ability to track changes in the inner apolar part of the membrane (DPH) or more superficial interaction with polar head groups of phospholipids (TMA-DPH) (**Figure 5**). When probes are exposed to a more polar aqueous environment the polarization is quenched.

It was observed that polarisation values were higher at 25ºC where lipids are in a more ordered and rigid state with lower fluidity. Oleuropein did not seem to significantly alter the membrane fluidity at this temperature. Above the T_m, presence of oleuropein at higher concentrations (> 1:1 molar ratio) increased polarization, meaning that interaction with phospholipids head groups may stabilize the membrane fluidity above the gel-to-liquid transition. This effect was more remarkable in samples with encapsulated oleuropein (not shown). Membrane lipids peroxidation was determined with BODIPY 581/591 C11 fluorescent probe and TBARS assay. Oleuropein showed a strong antioxidant potential to inhibit lipid peroxidation, and therefore promote membrane oxidation stability.

7. Conclusions and Future Perspectives

Due to the complexity of food matrices and encapsulating systems, optimisation and knowledge of the processing variables is needed in order to understand and predict behaviour of systems. As part of this PhD project, freeze-drying encapsulation of phenolic extract from olive leaves was optimised in terms of encapsulation efficiency as function of several processing variables showing that the physicochemical characteristics of wall material and the amount of core bioactive in relation to wall material play a major role.

Liposomes are biocarriers that can be manufactured in a cost-efficient manner for the food industry. However, their effectiveness and quality and stability can vary depending on their interaction with the compound of interest to be encapsulated. Therefore, it is important to understand how both components interact to predict their technofunctional and health potential. Liposomes can also be used as model membranes to study the potential antioxidant capacity to prevent free radical formation and lipid peroxidation, with implications for a food preservation and biological membranes.

This project will be completed with additional studies on extrusion and co-milling encapsulation, and incorporation of selected encapsulated ingredients in model food systems.

8. Funding grant

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