UNIVERSITA’ DEGLI STUDI DI SALERNO
Department of Industrial Engineering
Ph.D. Course in Chemical Engineering
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NANOENCAPSULATION OF BIOACTIVE COMPOUNDS FOR FOOD APPLICATIONS

Abstract

The increase in dietary-intake-related illnesses, such as obesity, cardiovascular diseases, hypertension, diabetes and cancer, have made in recent years the development of health-and-wellness promoting foods a priority of the food industry. Clinical studies have demonstrated tangible health benefits that may be derived from the intake of bioactive compounds. However many difficulties are associated with their inclusion in food matrices, due to a very low solubility in water and easy degradation by hostile environmental conditions once extracted from plant tissues. Furthermore, poor solubility also means lower absorption in the gastrointestinal tract and, therefore, limited bioavailability. In the food industry, it has become apparent that there is a pressing need for edible delivery system to efficiently encapsulate, protect and release bioactive compounds when developing functional foods.

This thesis was addressed to the study and engineering of nanoencapsulation systems, above all nanoemulsions and solid lipid nanoparticles, with superior capabilities of a) protecting the encapsulated bioactive compounds from interaction with food ingredients, keeping their functional properties and preventing the deterioration of the food itself (i.e. oxidation of fat), b) reducing the impact on the organoleptic properties of food and c) improving the absorption and bioavailability of the bioactives, due to the subcellular size of the nanocapsules, which may potentially enhance passive transport mechanisms (i.e. related to the concentration gradient) across the intestinal wall.

The research activity has contributed to the advance of the knowledge in the field of the science of colloids, through the specific investigation of the effects of formulation and process parameters, which influence nanoemulsion production, as well as to a deeper comprehension of the technological and biological aspects of the incorporation of the nanoencapsulated compounds in food matrices and explication of their activity.

Three different classes of bioactive compounds were chosen as model systems of the experimental work, namely curcumin, resveratrol and essential oils. Both curcumin and resveratrol are antioxidant compounds with markedly low solubility both in aqueous and lipid phase, hence requiring the development of specific formulations. In contrast, essential oils can be easily blended with oils, but require their diffusion through the aqueous phase to attack the cell membrane of microorganisms and act as antimicrobials.

Therefore, novel formulations were developed using a combination of hydrophilic (sugar ester, defatted soy lecithin, polysorbate 20) and lipophilic emulsifiers (soy lecithin, glycerol monooleate) to encapsulate resveratrol in peanut oil droplets and disperse it in aqueous systems at a concentration of 100 mg/L, ten times higher than the therapeutic blood concentration. In the same way, curcumin has been encapsulated in solid lipid particles, using stearic acid as lipid phase, at a maximum concentration 1600 times higher than the solubility of curcumin in water (0.6 mg/L).

Once the formulation was defined, the issue of the actual fabrication of the nanometric delivery systems was faced from a fundamental point of view. In particular, the production of food nanoemulsions by high pressure homogenization (HPH) has been investigated, focusing on the effect on droplet nanonization of emulsifier type and concentration, as well as of the geometry of the homogenization chamber. The reported results showed that the kinetic parameters of the emulsification process can be primarily correlated with the interfacial and dynamic properties of the emulsifiers, while the fluid-dynamics regime established in the homogenization chamber contributes only to a lesser extent. Nevertheless, the correct design of the homogenization chamber may help in obtaining uniform fluid-dynamic conditions, which ensure a narrow droplet size distribution.

The issues related to the physicochemical stability of nanoencapsulated bioactive compounds was faced for resveratrol and curcumin, trying to improve the formulation based on the inputs derived from accelerated ageing studies, that could simulate the food processing and the shelf life of the final product. The results obtained demonstrated that the nanoemulsions based on soy lecithin/sugar esters and Tween 20/glycerol monooleate can better encapsulate resveratrol in the lipid matrix, protecting it both during accelerated ageing and gastro-intestinal digestion and promoting a sustained release. Moreover, these formulations, having smaller mean droplet diameters (below 200 nm), remained physically stable also after the digestion process, allowing the resveratrol to reach the intestinal wall entrapped in the lipid droplets.

The subcellular dimension and the compatibility with cell membranes of the developed formulations also resulted in a higher permeability through the intestinal wall, which was simulated studying the transport through Caco-2 cell monolayers grown on permeable supports. Generally, the apparent permeability of most compounds falls in a range of 1×10^{-7} cm/sec (poorly transported compound) to 1×10^{-5} cm/sec (well-transported compound). The apparent permeability of resveratrol encapsulated in different nanoemulsion-based delivery systems resulted always in the range indicated, demonstrating that nanoencapsulation can improve passive transport mechanisms. In particular, soy lecithin/sugar esters-based formulation showed an higher permeability due to the presence of soy lecithin, which, having a structure similar to the phospholipid bilayers of the cellular membrane, favours the absorption and the entrapment of the oil droplets in the microvilli and their consequent transport through the cell membrane.

Another remarkable result of the present thesis is that for the first time the effect of the delivery systems on the antioxidant activity of nanoencapsulated compounds was investigated, using a biological-based approach that integrated the classical chemical approaches. More specifically, an improved cellular assay was developed, that enabled to measure exclusively the residual activity of nanoencapsulated bioactive that penetrated inside Caco-2 cells, giving precious information on the combination of the mass transfer promotion and protection by the delivery systems.

Finally, the technological issue related to the incorporation into fruit juices of essential oils encapsulated into nanometric delivery systems was investigated, having as goal the design of systems that are able to enhance antimicrobial activity of the bioactive compounds, while minimizing the impact on the quality attributes of the final product. The results showed that, due to the higher antimicrobial activity of the nanoencapsulated essential oils, lower antimicrobial concentrations are required for a bactericidal action with a minimal alteration of the organoleptic properties of the juice.

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