**Innovative technologies for the formulation of bioactive ingredients from plant-based by-products and development of functional foods**

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Lipid oxidation is the major challenge when developing functional foods and supplements containing vegetable oils rich in polyunsaturated fatty acids (PUFAs). Despite the recognized nutritional value of PUFAs, their physical and chemical characteristics still limit their application. Microencapsulation of PUFAs rich oils offers the possibility of obtaining lipophilic ingredient with enhanced stability and bioavailability. Accordingly, this PhD thesis project aims to investigate the encapsulation of polyunsaturated oils into different food-grade wall material, using a novel technology based on supercriticalcarbon dioxide, named particles from gas saturated solution (PGSS).

Tecnologie innovative per la formulazione di ingredienti bioattivi da sottoprodotti vegetali e sviluppo di alimenti funzionali

L'ossidazione dei lipidi è la sfida principale quando si sviluppano alimenti funzionali e integratori contenenti oli vegetali ricchi in acidi grassi polinsaturi (PUFAs). Nonostante il riconosciuto valore nutrizionale dei PUFAs, le loro caratteristiche fisiche e chimiche ne limitano ancora l'applicazione. La microincapsulazione di oli ricchi in PUFAs offre la possibilità di ottenere ingredienti con migliori proprietà funzionali e una prolungata stabilità all´ossidazione. Questo progetto di tesi di dottorato mira ad incapsulare oli ricchi in acidi grassi polinsaturi applicando una nuova tecnologia basata sull´utilizzo dell´anidride carbonica supercritica, denominata “particelle da una soluzione satura di gas (PGSS)”.

# **State-of-the-Art**

Consumer´s increasing interest in the health enhancing role of specific foods and physiologically active food components is having strong impact on the food industry. One of the major challenges is developing new ingredients that are healthy, functional, and natural (Bharat *et al.,* 2016). Vegetable oils are the main dietary source of essential fatty acids. They play an important role in the body satisfying nutritional needs. They are vital for the normal functioning of the brain and nerve system. The term essential fatty acid refers to polyunsaturated fatty acids (PUFAs) that cannot be produced by the human body and must be obtained from the diet (Kaur *et al.,* 2014). However, PUFAs have an unstable chemical structure and are susceptible to oxidation, isomerization, polymerization, and volatile component loss when exposed to different environmental stresses such as oxygen, light, moisture, and heat. This can lead to the formation of hydroperoxides and results in unpleasant flavours and odours (Jurić *et al.,* 2022). As a result, the health-benefit properties of PUFAs remain underused in formulated food products. Therefore, it is critical to develop suitable methods to improve the oxidative stability of PUFAs during processing and storage.

Microencapsulation is an established strategy for overcoming these challenges. The technology allows to formulate unstable oily molecules into free-flowing and stable powders, reducing oxygen access and providing good oxidation protection for the oil (Reis *et al.*, 2022). Several technologies such as spray drying, freeze drying, coacervation, and extrusion among others are often used to form solid lipid particles. Nevertheless, particle formation employing supercritical carbon dioxide (SC-CO2) has received increasing attention, as it operates at relatively low temperature and in an oxygen-free environment (Klettenhammer *et al.,* 2020). The Particles from Gas Saturated Solutions (PGSS) process is the most common example of encapsulation technology based on SC-CO2. The principle consists of saturating the supercritical fluid with the mixture made of the bioactive compound and the wall material. The saturated solution is then rapidly expanded though a nozzle at atmospheric pressure. The rapid release of CO2 causes an intensive cooling effect leading to the formation of solid or liquid particles. PGSS is a green technology that offers clear advantages, by using a non-flammable, non-toxic, abundant, cheap and generally recognized as safe solvent (SC-CO2) (Kravanja *et al.,* 2022). Moreover, it is suitable for lipophilic formulations (due to the apolar nature of CO2), and ensures gentle treatment for heat-sensitive bioactive compounds (Klettenhammer *et al.,* 2020).

# **PhD Thesis Objectives and Milestones**

The research objectives of this PhD project can be achieved through the following activities and working plan as shown in the Gantt diagram given in Table 1:

A1) **Microparticles production using PGSS**

 To evaluate the effectiveness of PGSS, for designing encapsulated ingredients (polyunsaturated fatty acids rich oils) using combination of different food-grade wall material, and assessing their loading capacity and oxidative stability.

A2) **Co-encapsulation of edible oils with natural (plant by-product) antioxidants**

 Although, the encapsulated oil is protected by the wall material, the addition of antioxidants can enhance its oxidative stability and offer advantages in producing powders with a variety of bioactive functionalities. Consequently, vegetable by-products will be extracted using SC-CO2, characterized, and added to the formulation.

A3) **Application or incorporation of these microparticles in food products**

 To understand if the claimed obtained physiochemical and functional properties are transferrable to food products, the encapsulated particles will be incorporated into food matrices to evaluate the functionality of the formulation.

A4) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

***Table 1*** *Gantt diagram for this PhD thesis project.*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Activity Months | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** |
| A1) | ***Microparticles production using PGSS***  |   |   |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 1) Process optimization |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 2) Powder characterization |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
| A2) | ***Co-encapsulation with natural antioxidants***  |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |   |  |   |   |   |  |  |  |  |
|  | 1) characterization of extracts  |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |  |  |  |   |   |  |  |  |  |
|  | 2) Oxidative stability assessment  |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |   |  |   |   |   |  |  |  |  |
| A3) | ***Incorporation in food products***  |   |  |   |   |   |   |  |   |   |   |   |   |  |   |  |  |  |  |   |   |   |   |   |  |
|  | 1) Effect of processing on the stability of the formulation |   |  |   |   |   |  |  |   |   |   |   |  |  |   |  |  |  |  |   |   |   |   |  |  |
| A4) | ***Thesis and Paper Preparation*** |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |  |   |   |   |   |   |   |   |

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