Design and development of meat and dairy analogues using vegetable proteins from upcycled sources

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This PhD research project is aimed at valorising alternative proteins extracted from plant by-products towards their use in the design and development of meat and dairy analogues. This general objective will be pursued starting from the characterization and improvement of the protein’s techno-functional properties, thus selecting the most promising ones for the optimization of formulations and processes to obtain safe, nutritionally valuable, and sensory acceptable products.

**Progettazione e sviluppo di analoghi della carne e di prodotti lattiero-caseari mediante l’impiego di proteine estratte da sottoprodotti di origine vegetale**

# Questo progetto di dottorato ha come scopo quello di valorizzare proteine alternative estratte da sottoprodotti vegetali mediante il loro utilizzo nella progettazione e nello sviluppo di analoghi della carne e di prodotti lattiero-caseari. Questo obiettivo generale sarà perseguito a partire dalla caratterizzazione e miglioramento delle proprietà tecnico-funzionali delle proteine studiate, selezionando quelle più promettenti per l'ottimizzazione di formulazioni e processi volti ad ottenere prodotti sicuri, nutrizionalmente validi e sensorialmente accettabili.

# **1. State-of-the-Art**

Over the past decade, the increase in the global population and the growing awareness towards the sustainability impact of livestock farming have generated a large demand for alternative eco-friendly proteins that can meet the nutritional needs of the population, without excessively impacting the ecosystem (Aiking and de Boer, 2020). To ensure a proper integration into the human diet, proteins should be safe, nutritionally valuable, and should be characterized by adequate functional and sensory characteristics that allow their inclusion in food formulations. In this regard, plant-based proteins have emerged as promising candidates, and their use is already widespread. Soybean, pea, and gluten are the most used raw material due to their availability, affordability, and high functionality. However, many other proteins sources are potentially suitable for these kinds of applications and are currently object of investigation. In this context, considering that approximately 14 percent of the food is globally lost or wasted along the agri-food chain between harvest and retail market (FAO, 2019), and that high quantities of this material are rich of proteins, plant by-products represent an interesting source of alternative proteins (Kamal et al., 2021). Utilization of plant proteins is not always straightforward; some strategies have been studied over time to improve their sensory, nutritional, and technological properties, including fermentation and high hydrostatic pressure (HPP) (Akharume et al., 2021; Avelar et al., 2021).

The demand for meat and dairy analogues is growing rapidly because of the shift toward plant-based diets due to concerns about human health, animal welfare, and environment (He et al., 2020). Developing such products is challenging due to the different structural and functional characteristics of animal-derived ingredients compared to the plant-based ones.

Plant-based alternatives to dairy products can be produced starting from mixture of isolated ingredients (such as water, plant proteins, vegetable oils, starch/hydrocolloids, salts, and starter cultures) or from whole plant material that can be soaked and then destroyed to form a colloidal structure. In both cases the result is a colloidal dispersion that can be homogenized and structured through different methods and processes, including enzymatic reactions, fermentation, acid/salt addition, and heat-gelation (Grossmann and McClements, 2021).

Plant-based meat analogues can include imitations of products such as hamburgers, sausages, patties, chicken nuggets, steaks, and more. A major challenge in the development of this type of product relates to the commonly globular structure of plant-proteins, strongly different if compared to the elongated fibrous shape of meat proteins. Therefore, technologies have been developed for texturizing plant proteins to give them a fibrous shape (e.g., high, and low moisture extrusion, shear cell technology, and electrospinning) (Baune et al., 2022).

This PhD project is part of the IPSUS project "Climate-smart food innovation using plant and seaweed proteins from upcycled sources" (ERA-NETs SUSFOOD2 and FOSC Joint Call) which is aimed at using innovative approaches to explore opportunities for upcycling plant and seaweed proteins from agri-food raw materials of different origins (pumpkin, hazelnut, grape, potato, brewers' spent grain, seaweeds).

The purpose of this PhD project is primarily, to investigate the techno-functional properties (e.g.: gel-forming capacity, emulsifying capacity, foam-forming capacity, solubility, water absorption capacity, oil absorption capacity), of alternative and under-studied proteins extracted from the selected by-products, and to test technological approaches aimed at their modification (HPP, Fermentation). The second objective will be to use the most promising alternative proteins for the formulation of alternative meat and dairy prototypes by testing different technologies and optimizing formulation and process parameters by a design of experiment approach.

# **2. PhD Thesis Objectives and Milestones**

Within the overall objective mentioned above, this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

1. **Literature research**.
2. **Characterization and modification of proteins techno-functional properties:** Characterization of the selected proteins techno-functional properties (Foaming, Emulsifying, Gelation, Solubility, WAC, OAC) **(A2.1);** testing the effect of conventional and alternative technologies (e.g., Fermentation, HPP) **(A2.2).**
3. **Design and development of plant-based cheese prototypes:** Selection of the most promising upcycled proteins (output of A2), formulation and optimization of the recipe, based on marketed products, scientific literature, and pre-testing **(A3.1);** Comparison and optimization of different technological unit operations for emulsifying (HPH) and structuring (Heat-treatment, acid/salt addition, fermentation) the ingredients **(A3.2);** Characterization of functional and sensory properties of the prototypes **(A3.3)**.
4. **Design and development of plant-based meat prototypes:** Selection of the most promising upcycled proteins (output of A2), formulation and optimization of the recipe, based on marketed products, scientific literature, and pre-testing **(A4.1);** Optimization of process parameters for protein texturization (high moisture extrusion) **(A4.2);** Characterization of functional and sensory properties of the prototypes **(A4.3)**.
5. **Thesis and papers writing.**

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

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Months** **Activity** | | **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) | ***Literature research*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2) | ***Protein functionality*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *1) Characterization* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *2) Modification* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3) | ***Cheese alternatives development*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *1) Formulation design* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *2) Process design* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *3) Characterization* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4) | ***Meat alternatives development*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *1) Formulation design* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *2) Process design* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *3) Characterization* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A5) | ***Thesis and papers preparation*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

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