Valorisation of Alternative Protein Sources by Tailored Biotechnological Processes and Non-Thermal Technologies to Obtain New Ingredients to Be Used in the Formulation of Innovative Foods

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This PhD thesis research project is aimed at obtaining new innovative ingredients from alternative protein sources using tailor made biotechnological processes and non-thermal technologies. After a careful selection of QPS microorganisms and matrices, the ingredients produced will be characterized and used to formulate innovative foods.

Valorizzazione di nuove fonti proteiche, attraverso processi biotecnologici di precisione e tecnologie non termiche, per l’ottenimento di ingredienti da utilizzare nella formulazione di alimenti innovativi

Questo progetto di tesi di dottorato mira all’ottenimento di nuovi ingredienti a partire da fonti proteiche alternative sfruttando processi biotecnologici e trattamenti non termici. Dopo un’attenta selezione di microrganismi con status QPS e delle matrici, gli ingredienti prodotti verranno caratterizzati e utilizzati per la formulazione di alimenti innovativi.

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

Due to the progressive world population increase, it is necessary to find new sources of food and develop new techniques for valorising existing resources. In 2021, world consumption of protein of animal origin stood at around 478 million tons, with differences that mainly depend on the geographical area, traditions and prices. According to FAO, the quantities of protein sources of animal origin produced in the world will not increase in the next ten years and the quantities consumed per capita are expected to remain almost constant. However, market demand will increase, mainly due to the increase in the population that needs these resources (FAO, 2021). Therefore, obtaining and valorising proteins derived from alternative sources is arousing more and more interest, both from industry and from research. Examples of new protein sources can be obtained from vegetables such as legumes, cereals and pseudocereals or waste and vegetable by-products from the food and feed industry (Molfetta *et al*., 2022). These ingredients are suitably functionalized using tailored biotechnological approaches based on microorganisms for which the European Food Safety Authority (EFSA) has recognized the qualified presumption of safety (QPS) and identified as GRAS (Generally Recognized as Safe) by the Food and Drug Administration (FDA), or non-thermal processes such as high homogenization pressures (HPH) and pulsed electric fields that allow the efficient extraction and valorisation of high-quality proteins. For example, bacteria belonging to the genus *Bacillus* have been used to valorise vegetable waste to obtain bioactive compounds; yeasts such as *Saccharomyces cerevisiae* and lactic acid bacteria have been used to ferment waste from the processing industry of cereals, fruit, vegetables and legumes, significantly increasing the amount of functional peptides in the extracted products. Recently many studies are also focusing on the use of unconventional yeasts such as *Debaryomyces* spp., *Kluyveromyces marxianus* and *Yarrowia lipolytica* to valorise waste and by-products of the agri-food industry (Gottardi *et al*., 2021). For example, *Y. lipolytica* has been used to valorise another alternative protein source such as insect meals (Molfetta *et al*., 2022). In fact, following the growth of this yeast on cricket flour, protein hydrolysates were obtained, with increased functionality and higher protein/peptide content, also used as ingredients in the production of bakery products (Patrignani *et al*., 2020; Rossi *et al*., 2022; Rossi *et al*., 2021). In general, biotechnological processes make it possible to obtain compounds starting from waste and by-products and from alternative protein sources that demonstrate better antioxidant, antihypertensive, antimicrobial, preservative and aromatic activity compared to those obtained with other enhancement techniques. Therefore, suitably functionalized proteins and by-products can be reused in traditional and/or innovative food formulations, in line with today's objectives of sustainability and circular economy. EFSA has already recognized the safety of some alternative proteins deriving from vegetable sources and fermented vegetable waste and by-products, identified as novel foods pursuant to regulation (EU) 2015/2283 and usable as innovative ingredients in food products.

# **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:

A1) **Bibliographic research** inherent to the topic, functional for the identification of the starting matrices (A1.2) and potentially exploitable microorganisms (A1.1), including unconventional yeasts, Bacilli and lactic acid bacteria.

A2) **Characterization of the identified strains and optimization of microbial performance** through the evaluation of the technological, functional and safety characteristics of the microorganisms (A2.1) in order to choose the most interesting for their enzymatic and metabolic activities.

A3) **Characterization of the matrices of interest** from a microbiological, nutritional and safety point of view (A3.1).

A4) **Obtaining and characterization of the ingredients** starting from the selected matrices and the use of the best performing microorganisms: identification of the most appropriate process conditions for the development of biotechnological processes on the selected matrices (as they are or non-thermally treated) (A4.1), characterization of innovative ingredients from safety, nutritional value and stability point of view (A4.2), their regulatory framework as established by EFSA (A4.3) and development of tailor-made protocols for their large-scale production.

A5) **Development of traditional or innovative products** using the most promising ingredients (A5.1) and characterization to evaluate their safety, microbiological shelf-life, quality, nutritional value and functionality (A5.2).

A6) **Writing and publication of the doctoral thesis, posters, scientific articles and oral presentation** (A6).

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

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Activity Months | | **2** | **4** | **6** | **8** | **10** | **12** | **14** | **16** | **18** | **20** | **22** | **24** | **26** | **28** | **30** | **32** | **34** | **36** |
| A1) | ***Bibliographic research inherent to the topic*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Alternative protein sources identification |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Safe and QPS microorganisms identification |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2) | ***Characterization of strains and optimization of microbial performance*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Evaluation of the technological, functional and safety characteristics  of selected microbial strains, microbial strains selection  and fermentative and technological performance optimization |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3) | ***Characterization of the matrices of interest*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Evaluation of microbiological, nutritional and safety  characteristics of selected main protein sources |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4) | ***Obtaining and characterization of ingredients*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1)Preparation of biotechnological processes and optimization of  process conditions (time, T, inoculum level). |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Evaluation of nutritional, stability and safety characteristics  of obtained ingredients |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 3) Regulatory framework |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A5) | ***Development of traditional or innovative products*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Traditional/innovative food formulation including  previously selected ingredients |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Characterization about safety, microbiological shelf-life,  quality, nutritional value and functional aspects |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A6) | ***Thesis and articles preparation and participation in conferences*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

# **3. Selected References**

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