**Biotechnological approaches for the valorisation of by-products from marginal areas**

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This PhD thesis research project aims to valorise waste, by-products and agro-industrial surpluses particularly abundant in marginal areas. The application of biotechnological processes, through the use of selected autochthonous microorganisms and/or coming from chemically similar matrices, will aim to increase the content of bioactive compounds present in the waste/by-product in order to produce functional foods and/or food supplements.

**Approcci biotecnologici per la valorizzazione di sottoprodotti delle aree marginali**

Questo progetto di tesi di dottorato mira a valorizzare scarti, sottoprodotti e surplus agro-industriali particolarmente abbondanti nelle aree marginali. L’applicazione di processi biotecnologici, attraverso l’uso di microrganismi selezionati autoctoni e/o provenienti da matrici chimicamente simili, avrà lo scopo di aumentare il contenuto di composti bioattivi presenti nello scarto/sottoprodotto al fine di produrre alimenti funzionali e/o integratori alimentari.

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

In recent years, the necessity of sustainable development due to environmental pollution and a continuous decrease in natural resources has become increasing (Martins de Olivera M.et al., 2021). Moreover, food security currently appears to be seriously threatened by an exponential demographic development that requires alternative food sources without exhausting the agricultural sector. To address this issue, the use of by-products as ingredients in food could be a possible strategy (Barreira J. et al., 2019).

This PhD-project aims to valorise waste, by-products and/or agro-industrial surpluses from marginal areas (like almond, asparagus, artichoke etc.), by applying technological and bio-technological approached to enhance the bioavailability of functional compounds. In detail, at the first literature research focused on waste from the almond processing industry. Firstly, the almond tree is present in the marginal areas as it resists to high temperatures, to drought conditions and in the presence of poor soils (Romero et al., 2004). Secondly, almond processing generates large quantities of waste, such as hull, shell and skin, which are not used in the food sector but are mainly used as feed for livestock or as fuel (Garcia-Perez P. et al., 2021). However, the food industries show greater interest in these by-products as it is enriched in bioactive compounds, such as polyphenols or unsaturated fatty acids (Barral-Martinez M.et al., 2021). In addition, by-products that are generated by the extraction of almond oil (almond press-cake) are particularly rich in proteins. The treatment of proteolytic enzymes of almond press-cake can lead to the production of bioactive peptides (de Souza et al., 2020). In a recent study by Pasqualone et al. (2020) almond skin was used to increase the nutritional and functional value of biscuits. However, there are no studies in the literature that investigated the application of fermentation process on almonds by-products. The application of fermentation processes could be a strategy to further enrich the waste into bioactive compounds (Sadh et al., 2018) or to make them more bioaccessible to produce fortified foods and/or food supplements.

**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 showed in Table 1:

A1) **Chemical and microbiological characterisation of processing by-products, waste and/or agro-industrial surpluses**: microbiological characterisation by colture-dependent methods.

A2) **Isolation and selection of Lactic Acid Bacteria and optimization of fermentation protocol:** isolation of "autochthonous" LAB starting from the same matrix or after a spontaneous fermentation process of waste/by-products/agro-industrial surpluses; LAB selection with strong fermentative (growth and acidification curve) and proteolytic activity. Development and optimization of the fermentation process by modulating temperature, duration and percentage of inoculum.

A3) **Characterisation of fermented by-products/waste/agro-industrial surpluses:** chemical and microbiological characterization and evaluation of health-promoting activities through faecal batches.

A4) **Production of fortified food with processed by-products/waste/agro-industrial surpluses.**

A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

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

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 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** |
| A0) | ***Literature search*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A1) | ***Chemical and microbiological characterization*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2) | ***Isolation and selection of Lactic Acid Bacteria and development of the fermentation protocol*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3) | ***Characterization of fermented waste/by-products/agro-industrial surpluses*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4) | ***Production of fortified food*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A5) | ***Thesis and Paper Preparation*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

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